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# *The Journal* *of* *Laboratory and Clinical* *Medicine*

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## VOLUME VII

OCTOBER, 1921—SEPTEMBER, 1922

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# *The Journal of Laboratory and Clinical Medicine*

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VOL. VII.

ST. LOUIS, OCTOBER, 1921

No. 1

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## ORIGINAL ARTICLES

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### STUDIES IN THE SEROLOGY OF SYPHILIS. SYPHILIMETRIC COLOR-INDICES\*

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(PRELIMINARY REPORT ON 317 CONSECUTIVE CASES)

BY ERNEST F. MAHR, WASHINGTON, D. C.

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#### PRELIMINARY CONSIDERATIONS

THE laboratory diagnosis of syphilis has been accomplished with more or less accuracy by a variety of methods. The majority of these methods are serologic in nature and depend upon the Bordet-Gengou phenomenon of complement fixation, while there are other methods, hitherto not of a thoroughly satisfactory character, which deal with the problem on a strictly chemical basis. Of the two types of methods those of a serologic nature are used extensively as routine tests for syphilis and have given fairly consistent results in spite of their complicated technic. The chemical tests, while far less complicated and more readily carried out, have shown an unfortunately low degree of specificity and a variable consistency with the physical diagnosis.

The chemical type of reaction is illustrated by the Bruck<sup>1</sup> reaction, in which nitric acid is the sole reagent employed. A positive reaction consists of the precipitation of certain albuminoids by the nitric acid, the degree of syphilitic infection being determined from the intensity of the precipitate. Bruck's reaction is specific for syphilis only to the extent of about 75 per cent, frequently giving positive reactions with sera showing a negative Wassermann. The lack in specificity of this reaction is perhaps due to the presence in the blood serum of several closely related albuminoids, most of which will precipitate with the nitric acid, but only one or two of which are the substances that are specific for syphilis. Inasmuch as the chemical structure of the syphilitic antibody is as yet undetermined, the test for its presence by a strictly chemical reaction would appear to be more or less experimental in character, and hence of doubtful practical value.

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The serologic methods for the diagnosis of syphilis fall essentially into two groups, both of which depend upon complement fixation. Methods of the first group follow the original Wassermann reaction in that they employ complement and amboceptor each of which is derived from a separate source. Methods of the second group employ either complement or amboceptor, or both, present in a native state in the serum of the patient.

Illustrative of the first group is the original method of Wassermann, probably actually due to Neisser, employing guinea pig complement and the serum of a rabbit immunized against sheep erythrocytes as amboceptor; and the Noguchi<sup>2</sup> modification in which human erythrocytes and a corresponding antihuman rabbit amboceptor are used. Aside from these essential differences there are, of course, minor differences in the details of the technic employed, such as the paper amboceptor used by Noguchi for example.

In the second group may be given the Hecht-Weinberg-Gradwohl<sup>3</sup> reaction, in which is used both the complement and amboceptor native in the patient's serum; and Wright's<sup>4</sup> modification of the Wassermann in which both native complement and amboceptor are employed. There is also a recent method given by G. A. Magnusson<sup>5</sup> in which the native complement alone is used, the amboceptor being obtained from a separate source.

A great difficulty encountered in all forms of the Wassermann reaction is the variable nature of nearly all the reagents. These reagents, whose chemical structure is practically unknown, do not possess the stability of chemical reagents, but are subject to alteration and deterioration from the standpoint of temperature, age and bacterial activity, and consequently cannot be adequately standardized, or preserved conveniently. It appears essential, therefore, to utilize fully what means there may be for keeping these reagents in as uniform a condition as possible, and to eliminate as many of the variable features as possible.

Comparing, with these points in mind, the two groups of methods described above, it is evident that the first group has certain advantages over the second. Whereas, for example, in the Hecht-Weinberg-Gradwohl method one must be satisfied with the variable amounts and proportions of native complement and amboceptor present in each particular serum, it is possible to titrate with good accuracy the guinea pig complement against the fairly stable rabbit amboceptor, as is done in the methods of the first group. Although the complement of one guinea pig may differ in titre from that of another, the same lot of reagent is used for a large number of tests at a time and hence it is possible to obtain results which are fairly comparable.

Another factor of importance, applying to all of these methods, is the manner in which the final results are interpreted. The routine procedure is to designate the final readings as one-, two-, three-, or four-plus, or negative, as the case may be. These readings give only a general indication as to whether a reaction is strongly or weakly positive. For the purpose of following the effects of antisyphilitic treatment upon the serology of a patient these designations are of little value. To obtain more accurate estimates of slight variations in the amount of syphilitic antibody present, it is necessary to make the final readings



upon a quantitative basis. If the quantity of sheep cells, complement and amboceptor used in the Wassermann reaction, is kept constant, then the amount of syphilitic antibody present in a serum is directly proportional to the number of cells left intact at the end of the incubation period, or inversely proportional to the degree of hemolysis at the end of that time.

Approaching the problem from the fact of the direct proportion of the number of cells to the amount of syphilitic antibody, methods have been devised for measuring the amount of nonhemolyzed cells. The great difficulty here, however, is the small amount of cells one has to deal with in the first place. It is extremely difficult to construct a graduated tube in which the amount of cells can be determined after centrifuging. Furthermore, the length of time of centrifuging and the speed of the centrifuge introduce additional sources of error.

The prospect appears more hopeful when the problem is viewed from the other point of view, that is to say, the inverse ratio of the degree of hemolysis to the amount of syphilitic antibody.

A. Vernes<sup>6</sup> has devised a method for accomplishing this by the use of an artificial color-scale which is composed of eight shades or "indices" ranging from that of complete hemolysis of a standard dose of sheep cells to the point of complete inhibition of hemolysis. Index "8" on this scale represents complete hemolysis, and "0" represents complete absence of hemolysis, while the intermediate tints correspond to successive increases in syphilitic antibody.

To carry out the reaction Vernes employs an antigen which he terms "perethynol," made by extracting horse heart with ethylene-perchloride and then alcohol. A suspension of this in physiologic salt solution constitutes a colloid which is flocculated in the presence of syphilitic antibody, in a manner similar to the precipitation of various inorganic and organic colloidal suspensions by both negative and positive sera. In place of the amboceptor and complement he uses hog serum. This acts as an "antiflocculant," and also possesses hemolytic properties for sheep cells. In exercising its antiflocculant activity the hog-serum loses a proportionate amount of its hemolytic property upon the sheep cells, and hence gives a direct index of the amount of syphilitic antibody in terms of hemolysis. Vernes gives this method the name of "Syphilimetric."

The method described here is the result of numerous experiments concerned with the proper dosage of the reagents and with the preparation of a colorimetric scale adapted to these dosages. The method was then carried out on a number of successive cases which were first subjected to the routine Wassermann test used at this laboratory, which is a modification due to Dr. Nolan D. C. Lewis, of this hospital. A satisfactory check was thus obtained for each individual specimen examined. The Lange colloidal gold reaction as well as the Wassermann was performed on each specimen of cerebrospinal fluid before it was tested by the colorimetric method.

In order to devise an adequate color-scale it was necessary to employ as high a concentration as possible of complement and amboceptor, and yet one low enough to remove the possibility of any interference from the color of the reagents themselves. In keeping with the strength of these reagents the sheep-cell suspension was prepared of such a strength that it was easily and invariably

hemolyzed by the doses of complement and amboceptor used. The concentration of sheep-cell suspension giving the best results was found to be practically three times that used for the sheep-cells in the routine Wassermann, that is, approximately 15 per cent. For use with the colorimetric scale the sheep-cells must be of such concentration that one dose, here .25 c.c., in the total volume represented by all the reagents in one tube, namely 1.5 c.c., when completely hemolyzed, shall correspond in tint with "8" on the scale, that is, shall give an index of "8." This is the index given by a completely negative serum. Index "7" is slightly positive, "6" more so, and from this point on there is a gradual increase in positiveness up to and including "0," which represents complete inhibition of hemolysis. Generally speaking, the color indices from 0 to 2 would be theoretically equivalent to the usual designation of four-plus, 2 to 4 would correspond to three-plus, 4 to 6 to two-plus, and any reading between 6 and 8 except 8 would be about one-plus. It is apparent from the results obtained, however, that no definite relation such as the foregoing exists. For example, any reading having a value lying between 0 and 5 on the colorimetric scale cannot readily be interpreted other than four-plus by the routine method. In making possible the gradation of such interpretations into finer and more distinct readings lies the value of the colorimetric scale.

A great deal of variation is also introduced into the routine Wassermann procedure by the manner in which the red-cell suspension is prepared. Ordinarily the cells are washed two or three times, and after a final centrifugalization they are arbitrarily taken to be at a concentration of 100 per cent. With this assumption a 5 per cent or 10 per cent suspension in physiologic saline is then prepared by adding, volumetrically, the proper amount of the arbitrary 100 per cent suspension to the correct amount of saline to give the percentage desired. The concentration of such a suspension cannot reasonably be expected to be uniform from time to time. Errors are introduced by the length of time of centrifugalization, the speed of the centrifuge and the amount of supernatant salt solution removed from the sedimented cells after the final washing.

These objectionable features are eliminated by the colorimetric standardization as is shown in the descriptions following. This part of the procedure might therefore be of value in standardizing the red-cell suspension for the usual Wassermann methods, even if no color scale is to be employed.

#### PROCEDURE

##### I. *Apparatus and Reagents.*—

The serologic tubes and flasks used are of hard glass with a low coefficient of expansion. All glassware is treated with bichromate-sulphuric cleaning mixture and washed in tap water followed by repeated rinsings in distilled water. This is followed by drying in a hot-air oven.

The reagents are prepared in volumetric glass-stoppered cylinders and flasks. The pipettes found most suitable are of 1 c.c. capacity graduated to deliver 1 c.c. These can easily be marked off at .25 c.c. levels, making the manipulation easier.

The antigens used in both the colorimetric method and the Wassermann check are of two types: A simple alcoholic extract of human heart, and a similar

alcoholic extract containing .2 per cent cholesterin. The cholesterinized antigen alone was used in the colorimetric method because of the greater dosage of patient's serum used. Both antigens were kept in stock containers at room temperature and have given satisfactory results for a period of at least two years.

The amboceptor consists of sterile rabbit serum, the animal having been immunized by repeated inoculations with a suspension of sheep erythrocytes. The serum was stored in 1 c.c. sealed ampoules in a low-temperature refrigerator.

Complement is obtained within 24 hours of each test by bleeding one or more guinea pigs from the heart and allowing the blood to clot in sterile centrifuge tubes at room temperature for about 3 hours and then overnight in the refrigerator after the clots are loosened.

Sheep erythrocytes are obtained within 24 hours of the test from the jugular vein and clotting is prevented by receiving the blood in a 1 per cent solution of sodium citrate in .85 per cent salt solution. The cells are washed by centrifugalization three times, for 5 minutes at a time, using speed "5" on an electric centrifuge.

The patients' serum is received in serologic test tubes, separated from the clots, diluted with 4 parts of .85 per cent sodium chloride solution and inactivated at 57° C. for 30 minutes, within 24 hours of each test. Spinal fluids which have been standing in the refrigerator for more than 24 hours are also inactivated.

A complement-amboceptor titration is done immediately before each test and appropriate controls are performed with all the reagents, both for the colorimetric method and the routine Wassermann. It is not regarded as essential to go into the details of these controls and titrations since they conform in type with the methods used by all syphilographers. An outline of the Wassermann method used as check, and of the colorimetric method will be given.

## II. Procedure for Routine Wassermann.—

After the preliminary titrations are performed with satisfactory results, a set of tubes is prepared as follows for each test.

TABLE I

TUBE NUMBER	PATIENT'S SERUM 20%	ANTIGEN "A"	"B" (choles.) ANTIGEN	COMPLEMENT 10%
I (control)	0.5 c.c.	0	0	0.25 c.c.
II	0.25	0.25	0	0.25
III	0.25	0	0.25	0.25

These tubes are incubated at 37° C. for 30 minutes. Then 0.5 c.c. of a mixture of equal parts of amboceptor (concentration used is determined in the preliminary titration) and 5 per cent sheep cells is added to each tube. The tubes are thoroughly shaken and incubated once more for 30 minutes at 37° C. Readings are then made.

For the routine Wassermann on cerebrospinal fluid the arrangement shown in Table II is used for each specimen.

The incubation period is 30 minutes at 37° C. Then .5 c.c. of the sensitized sheep cells is added to each tube. The second incubation is the same as the first. Readings are then made at once.

TABLE II

TUBE NUMBER	SPINAL FLUID (undiluted)	ANTIGEN "A"	ANTIGEN "B"	COMPLEMENT 10%
I (control)	.5 c.c.	0	0	.25
II	.25	.25	0	.25
III	.25	0	.25	.25
IV	1.00	0	0	.25
V	.5	0	.25	.25
VI	1.00	0	.25	.25

### III. Procedure for Colorimetric Method.—

Tubes are set up as shown in Table III for each specimen.

TABLE III

TUBE NUMBER	PATIENT'S SERUM 20%	ANTIGEN B (chol.)	COMPLEMENT 10%	.85% NaCl SOLUTION
I (control)	.5 c.c.	0	.25	.25
II	.5	.25	.25	0

The first incubation is 30 minutes at 37° C. Then .5 c.c. of sensitized cells are added to each tube. These cells are first standardized colorimetrically as described below. The second incubation is the same as the first.

Readings: All frankly negative reactions are recorded at once. All slightly turbid tubes are centrifuged, as well as all the strongly turbid tubes, for 5 minutes at speed "5." These tubes are then compared with the tubes containing the artificial color standards in 1.5 c.c. amounts, and the color index of each is noted. No reading is recorded unless the corresponding control tube gives an index of "8."

The procedure for cerebrospinal fluid is identical with that described for blood serum. The use of various amounts of spinal fluid is not adaptable to colorimetric determinations.

### IV. Standardization of Erythrocyte Suspension.—

After the final washing of the sheep cells the set of tubes as shown in Table IV is prepared:

TABLE IV

TUBE	SHEEP CELLS CENTRIFUGED	WATER DISTILLED	TOTAL VOLUME
I	.3 c.c.	1.2 c.c.	1.5 c.c.
II	.25	1.25	"
III	.20	1.30	"
IV	.15	1.35	"
V	.10	1.40	"
VI	.05	1.45	"

After a few minutes the cells in all tubes show complete hemolysis. From this set of tubes is selected the one whose tint corresponds with index "8" on the color scale. To prepare the suspension containing the corresponding dose in each .25 c.c., the following procedure is carried out:



Ex: If tube #6 gives index "8," then

- (1) To .05 c.c. sheep cells add .2 c.c. of .85 per cent salt solution.
- (2) or to .5 c.c. sheep cells add 2 c.c. of .85 per cent salt solution.
- (3) or to 5 c.c. sheep cells add 20 c.c. of .85 per cent salt solution.

#### V. Preparation of the Colorimetric Scale.—

The stock solution corresponding to Verne's "Liquid 8" is made up as follows:

0.1 per cent acid fuchsin in water	10 c.c.
1.0 per cent picric acid in water	10 c.c.

To the above is added 110 c.c. of the following acetic acid diluting mixture:

Acetic acid (glacial)	8 c.c.
Formalin 40%	5 c.c.
Water, distilled, enough to make	200 c.c.

From this stock solution a set of 9 tubes is prepared by dilution with acetic mixture, as shown in Table V.

TABLE V

TUBE	INDEX	STOCK LIQUID "8"	ACETIC MIXTURE	RATIO
1	8	1.0 c.c.	1.0 c.c.	1/1
2	7	1.0	2.0	1/2
3	6	1.0	3.5	1/3.5
4	5	1.0	5.75	1/5.75
5	4	.5	4.6	1/9.2
6	3	.5	7.1	1/14.2
7	2	.2	3.5	1/17.5
8	1	.2	4.5	1/21.8
9	0	.1	4.35	1/43.5

When properly prepared, the color tint of these standards has a perfect resemblance to the corresponding tints of actually hemolyzed sheep cells.

Attention is called to an interesting fact in connection with the dilution-ratios of the color scale. When the values for the acetic diluting mixture in each tube are plotted against the color indices from "0" to "8," it is found that a logarithmic type of curve is obtained. (Fig. 1.) This curve is produced by the fact that the increment in the quantity of diluting fluid from one tube to the next, beginning with index "8," is not uniform, but increases. This is shown in the denominators of the ratios in Table V. This increase in increment is necessary to bring about a uniform division into eight color tints. If the increments are made uniform, the tints of Tubes 8, 7, 6 and 5 can be differentiated only with difficulty, then there is a sudden drop to a lighter tint in Tube 4, and the remaining group of tubes shows very slight differentiation.

From this it follows that the color tints vary from "8" to "0" at the rate of the logarithms of their dilution-ratios.

The stock solution of Liquid "8" is quite permanent if kept in the dark. No appreciable deterioration in color value was found after nine months' standing when compared with a freshly prepared solution. The solution in the tubes of the color scale keeps equally well if the tubes are of hard glass and are carefully cleaned before being filled, and are then well sealed.

The comparison of tests with the standard tubes is facilitated by the use of a color-comparison box such as is shown in Fig. 2.

The tube whose index is to be determined is placed into the middle hole and tubes of a higher and lower index from the color scale are placed on either side of it. In this way close approximations are made possible. If a specimen of serum has itself a marked tint, a tube containing one dose of serum diluted to

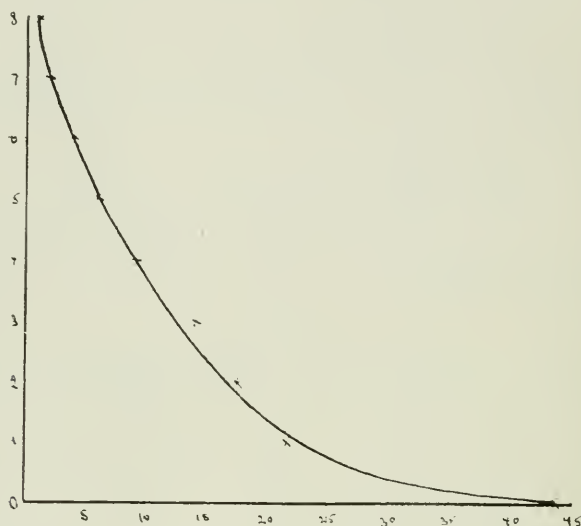


Fig. 1.—Color indices plotted against dilution ratios of color scale.

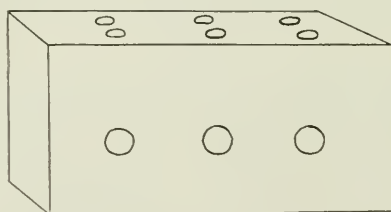


Fig. 2.—Color comparison box.

standard volume is placed behind the standards and the tube containing the test to be determined.

It is essential to use tubes of good quality and especially tubes of uniform diameter both for the color standards and for carrying out the reactions.

#### DISCUSSION OF TABLES

In Table VI is given a detailed list of the specimens of blood-serum examined by both methods.

In Table VII the specimens of cerebrospinal fluid are listed in the same manner. The colloidal gold curve of each specimen is also given.

Table VIII deals with the total number of 259 blood sera examined. It is divided into the whole scale of indices that may be obtained. Under index "8," for example, it is found that out of the 259 sera 200 gave index "8." In the

TABLE VI  
 COMPLETE RECORD OF BLOOD SPECIMENS

SERUM NUMBER	WASSERMANN	COLOR INDEX	SERUM NUMBER	WASSERMANN	COLOR INDEX
1	2 plus	2	61	0	8
2	0	8	62	0	8
3	0	8	63	0	8
4	4 plus	1	64	0	8
5	4 plus	3	65	0	8
6	2 plus	4	66	0	8
7	0	7	67	0	8
8	0	5	68	4 plus	0
9	0	8	69	0	8
10	0	8	70	0	8
11	0	8	71	0	8
12	0	8	72	0	8
13	0	8	73	0	8
14	0	8	74	0	6
15	3 plus	5	75	0	8
16	0	8	76	0	8
17	0	8	77	0	8
18	0	8	78	0	8
19	0	6	79	0	8
20	0	8	80	0	8
21	4 plus	12	81	0	8
22	2 plus	1	82	0	8
23	0	8	83	0	8
24	0	8	84	0	8
25	0	4	85	0	8
26	4 plus	1	86	0	8
27	0	8	87	0	8
28	4 plus	1	88	0	8
29	2 plus	5	89	4 plus	8
30	0	8	90	0	8
31	2 plus	6	91	0	8
32	0	8	92	4 plus	3
33	anticomp.	8	93	0	8
34	4 plus	0	94	0	8
35	2 plus	8	95	0	8
36	0	8	96	0	8
37	0	8	97	3 plus	8
38	anticomp.	8	98	0	8
39	0	8	99	5 plus	8
40	0	8	100	0	8
41	0	8	101	0	8
42	3 plus	7	102	0	8
43	4 plus	5	103	0	8
44	0	8	104	0	8
45	0	8	105	0	8
46	0	8	106	0	8
47	0	8	107	0	8
48	0	8	108	0	8
49	not done	7	109	4 plus	0
50	" "	7	110	0	8
51	0	8	111	4 plus	0
52	0	8	112	4 plus	0
53	0	8	113	0	8
54	0	8	114	4 plus	0
55	0	8	115	0	8
56	0	8	116	0	8
57	0	8	117	0	8
58	0	8	118	3 plus	5
59	0	8	119	0	8
60	0	8	120	4 plus	0

TABLE VI (CONTINUED)  
COMPLETE RECORD OF BLOOD SPECIMENS

SERUM NUMBER	WASSERMANN	COLOR INDEX	SERUM NUMBER	WASSERMANN	COLOR INDEX
121	4 plus	6	181	0	8
122	4 plus	0	182	2 plus	5
123	4 plus	0	183	0	8
124	0	8	184	2 plus	8
125	4 plus	0	185	0	8
126	4 plus	0	186	0	8
127	0	8	187	0	8
128	0	8	188	4 plus	6
129	0	8	189	0	7
130	0	8	190	0	8
131	0	8	191	0	8
132	0	8	192	0	8
133	0	8	193	2 plus	0
134	0	8	194	2 plus	6
135	0	8	195	2 plus	6
136	0	8	196	0	8
137	0	8	197	0	8
138	0	8	198	0	8
139	0	8	199	0	8
140	0	8	200	4 plus	8
141	0	8	201	0	8
142	0	8	202	0	8
143	0	8	203	0	8
144	0	8	204	0	8
145	0	8	205	0	8
146	0	8	206	0	8
147	0	8	207	4 plus	0
148	0	8	208	0	8
149	0	8	209	0	8
150	0	7	210	4 plus	7
151	4 plus	0	211	0	8
152	0	8	212	0	8
153	0	8	213	0	7
154	3 plus	0	214	anticomp.	8
155	plus minus	5	215	0	8
156	0	7	216	0	8
157	0	8	217	0	8
158	0	8	218	0	8
159	0	8	219	0	8
160	0	5	220	0	8
161	0	8	221	0	8
162	0	8	222	0	8
163	0	8	223	0	8
164	0	7	224	0	8
165	0	8	225	0	8
166	0	8	226	1 plus	8
167	0	8	227	0	8
168	0	8	228	0	8
169	0	8	229	0	8
170	0	8	230	4 plus	0
171	2 plus	7	231	4 plus	6
172	3 plus	7	232	0	8
173	0	8	233	0	8
174	0	8	234	1 plus	8
175	0	8	235	0	8
176	0	8	236	0	8
177	0	8	237	0	8
178	0	8	238	0	8
179	0	8	239	0	8
180	0	8	240	0	8



TABLE VI (CONTINUED)  
 COMPLETE RECORD OF BLOOD SPECIMENS

SERUM NUMBER	WASSERMANN	COLOR INDEX	SERUM NUMBER	WASSERMANN	COLOR INDEX
241	0	8	251	0	8
242	0	8	252	0	8
243	0	8	253	0	8
244	0	8	254	0	8
245	0	8	255	0	8
246	0	8	256	2 plus	6
247	0	8	257	0	8
248	0	8	258	4 plus	0
249	0	8	259	3 plus	5
250	0	8			

 TABLE VII  
 COMPLETE RECORD OF CEREBROSPINAL FLUIDS

FLUID NUMBER	WASSERMANN	COLOR INDEX	COLLOIDAL GOLD CURVE
1	4 plus	0	5 5 5 5 4 0 0 0 0 0
2	4 plus	6	1 1 1 1 1 1 0 0 0 0
3	0	8	normal curve
4	4 plus	0	5 5 4 4 2 0 0 0 0 0
5	4 plus	0	5 5 4 4 3 2 0 0 0 0
6	4 plus	6	5 5 5 4 4 3 0 0 0 0
7	0	8	normal curve
8	4 plus	3	2 2 1 1 0 0 0 0 0 0
9	4 plus	6	5 5 5 4 2 1 0 0 0 0
10	2 plus	8	5 4 4 2 2 0 0 0 0 0
11	4 plus	0	5 5 4 3 1 0 0 0 0 0
12	0	8	normal curve
13	0	8	normal curve
14	3 plus	8	normal curve
15	4 plus	7	3 4 5 5 5 4 3 2 1 0
16	0	8	normal curve
17	0	8	normal curve
18	3 plus	7	0 0 0 1 0 0 0 0 0 0
19	0	8	normal curve
20	4 plus	0	5 5 5 5 5 5 4 1 0 0
21	4 plus	0	5 5 5 5 5 4 3 1 0 0
22	4 plus	7	5 5 5 5 5 5 4 2 1 0
23	0	8	normal curve
24	4 plus	0	5 5 5 5 5 5 2 1 0 0
25	0	8	normal curve
26	4 plus	6	4 5 5 5 4 4 5 3 2 1
27	0	8	1 2 2 1 1 0 0 0 0 0
28	0	8	normal curve
29	4 plus	0	5 5 5 5 5 3 2 1 0 0
30	0	8	1 3 3 3 2 2 2 1 1 1
31	0	8	normal curve
32	3 plus	6	5 5 5 4 3 3 2 1 0 0
33	0	8	normal curve
34	0	8	normal curve
35	0	8	normal curve
36	4 plus	0	5 5 5 4 3 2 1 1 4 0
37	4 plus	0	4 2 3 3 2 1 0 0 0 0
38	4 plus	0	5 4 3 2 1 0 0 0 0 0
39	0	8	normal curve
40	0	8	normal curve
41	0	8	normal curve
42	0	8	normal curve
43	0	8	normal curve
44	3 plus	7	5 5 5 4 4 3 2 1 0 0

TABLE VII (CONTINUED)

FLUID NUMBER	WASSERMANN	COLOR INDEX	COLLOIDAL GOLD CURVE
45	3 plus	7	5 5 5 5 4 3 2 1 0 0
46			
47	0	8	normal curve
48	0	8	normal curve
49	0	8	normal curve
50	4 plus	0	5 5 5 5 5 4 3 2 1 0
51	4 plus	1	5 5 4 3 2 1 0 0 0 0
52	3 plus	6	4 3 2 1 0 0 0 0 0 0
53	2 plus	7	normal curve
54	anticomp.	8	normal curve
55	4 plus	0	5 5 5 5 3 2 1 0 0 0
56	4 plus	0	5 5 5 4 3 2 1 0 0 0
57	3 plus	7	5 5 5 4 3 2 1 0 0 0
58	3 plus	8	5 5 4 3 2 1 0 0 0 0

TABLE VIII

RELATION OF SYPHILIMETRIC INDEX TO THE WASSERMANN REACTION IN  
259 SPECIMENS OF BLOOD SERUM

INDEX	NUMBER OF SPECIMENS	WASSERMANN NO. OF SPECIMENS		PERCENTAGE OF SPECIMENS WITH	
		POS.	NEG.	POSITIVE WASSERMANN	NEGATIVE WASSERMANN
"8"	200	9	191	4%	96%
"7"	14	6	8	43%	57%
"6"	9	7	2	78%	22%
"5"	9	7	2	78%	22%
"4"	2	1	1	50%	50%
"3"	2	2	0	100%	0%
"2"	2	2	0	100%	0%
"1"	4	4	0	100%	0%
"0"	17	17	0	100%	0%

TABLE IX

RELATION OF SYPHILIMETRIC INDEX TO THE WASSERMANN REACTION IN  
58 SPECIMENS OF SPINAL FLUID

INDEX	NUMBER OF SPECIMENS	WASSERMANN NO. OF SPEC.		COLLOIDAL GOLD NO. OF SPEC.			PERCENTAGE WASSERMANN	
		POS.	NEG.	NEG.	PARETIC	SYPH. ZONE	POS.	NEG.
"8"	29	3	26	25	3	1	10%	90%
"7"	7	7	0	2	1	4	100%	0%
"6"	6	6	0	1	4	1	100%	0%
"5"	—	—	—	—	—	—	—	—
"4"	—	—	—	—	—	—	—	—
"3"	1	1	0	0	1	0	100%	0%
"2"	—	—	—	—	—	—	—	—
"1"	1	1	0	0	1	0	100%	0%
"0"	14	14	0	0	14	0	100%	0%

third and fourth columns of the same row is stated how many of these 200 specimens gave a positive and how many a negative routine Wassermann. These relations are then expressed in percentages in the fifth and sixth columns.

It is apparent from Table VIII that the percentage of positive routine Wassermans agrees with fair regularity with the color-indices obtained and that the percentage of positives shows a uniform increase from index "8" to index

TABLE X

 COMPARISON OF SYPHILIMETRIC INDEX AND ROUTINE WASSERMANN REACTION IN CASES  
 UNDER TREATMENT FROM FEB. 16, TO APRIL 24, 1921

## BLOOD SERUM

SPECIMEN	DATE TESTED	WASSERMANN	INDEX	TREATMENT	
				DOSES SALVARS.	DATE
M. R.	2-16-21	4 plus	1	.3g	2-14-21
"	3- 2-21	0	8	.4g	2-21-21
"	3- 9-21	4 plus	0	.4g	2-29-21
"				.4g	3- 7-21
B. K. R.	2-16-21	0	8	.15g (S)	2-14-21
"	3- 2-21	0	8	.2g (S)	2-21-21
"	3-24-21	0	7	.2g (S)	2-28-21
"				.1g (S)	3-21-21
P.	2-16-21	2 plus	4	.3g	3-24-21
"	3-30-21	0	7		
H.	2-23-21	antcomp.	8	.4g	2- 9-21
"	3- 2-21	0	8	.4g	2-16-21
"				.3g	3- 2-21
Par.	2-23-21	2 plus	8	no treatment	
"	3- 2-21	0	8	" "	
T.	2-23-21	3 plus	7	no treatment	
"	3- 2-21	0	8	" "	
"	3- 9-21	4 plus	7	" "	
Pe.	3-16-21	3 plus	5	.3g	3-24-21
"	3-30-21	0	5		

(S) = SILVER SALVARSAN

## SPINAL FLUID

SPECIMEN	DATE TESTED	WASSERMANN	INDEX	TREATMENT	
				DOSES SALVARS.	DATE
T.	3- 1-21	4 plus	0	.4g	2-23-21
"	3- 8-21	3 plus	8	.4g	3- 2-21
"	4-20-21	3 plus	8	.4g	3- 9-21
"				.4g	3-16-21
"				.4g	3-23-21
"				.4g	3-30-21
R.	3- 1-21	4 plus	6	.2g (S)	3-28-21
"	3-24-21	4 plus	6	.2g (S)	4- 4-21
"	4- 5-21	4 plus	7	20 c.c. (SE)	4- 5-21
Re.	3- 1-21	4 plus	3	no record	
"	3- 8-21	0	8	" "	

(S) = SILVER SALVARSAN

(SE) = SWIFT-ELLIS

"0," as would be expected. The fact that these specimens were not selected but were taken in the routine order of examination in the institution accounts for the large number of negatives.

In Table IX similar data is given for the cerebrospinal fluids, with the addition, of course, of the gold curves. Since the specimens of spinal fluid examined are for the most part from suspicious cases only, a high percentage of positives is found here. Attention is called to two findings shown in this table; first, the distinct predominance of the syphilitic zone type of curve in the higher indices, that is, from "6" to "8," and the simultaneous predominance of the parietic curves in the lower indices, that is, from "0" to "6"; second, the absence of readings for the indices of "2," "4," and "5." The latter fact is probably due,

TABLE XI  
SUMMARY OF 317 SPECIMENS EXAMINED BY THE  
COLORIMETRIC METHOD

BLOOD SERUM	
Total Number of Specimens	= 259
Positives (colorimetric method)	= 60
Positives (routine Wassermann)	= 54
Negatives (colorimetric method)	= 199
Negatives (routine Wassermann)	= 205
CEREBROSPINAL FLUID	
Total Number of Specimens	= 58
Positives (colorimetric method)	= 29
Positives (routine Wassermann)	= 32
Positive Curves, Colloidal Gold	= 29
Negatives (colorimetric method)	= 29
Negatives (routine Wassermann)	= 26

in part, to the fact that the total number of specimens of spinal fluid examined to date is not large.

Table X includes a number of cases receiving salvarsan, silver-salvarsan, and salvarsanized serum intraspinally, during the period of time covered by the tests made. The doses given and the dates of treatments are found in the last column. This table is included more by way of introduction than for the purpose of drawing definite conclusions, and more detailed data covering a greater period of time will be published subsequently.

#### SUMMARY

I. The colorimetric scale makes it possible to determine with fair accuracy the finer gradations in the degree of syphilitic infection. Indices from 1 to 4 inclusive, for example, cannot be recorded other than "4-plus" by the regular Wassermann procedure.

II. Doubtful readings, such as "plus-minus," 1-plus, and 2-plus are given a definite designation and are determined with greater certainty by the use of the color scale, and the personal equation of the observer is practically eliminated.

III. In the case of subjects under antisyphilitic treatment, the recognition of a slight drop toward the negative side is possible with the use of the color scale, and practically impossible with the routine Wassermann. Slight increases to the positive side are recognizable in the same manner.

IV. With paretic spinal fluids it is seen that the syphilimetric index approaches the reading of "0" in proportion as the colloidal gold reaction shows a definite paretic curve, whereas the routine Wassermann reading will give simply a "4-plus" reading whether the curve is of a strong paretic type or not. The syphilitic zone of gold curve predominates in the region of indices from "6" to "8," and the paretic type in the region from "6" to "0."

V. Colorimetric readings make possible the recording of the laboratory history of syphilis for each patient upon a graphic chart, giving a clear representation of the progress of cases under treatment. In Fig. 3 is suggested a method for the drawing of charts.

## APPLICATION OF VERNES' METHOD TO THE WASSERMANN REACTION

In the method of Vernes a suspension of an alcoholic lipoidal extract of horse heart is used. This colloidal suspension, which is of the same chemical character as the antigens generally employed in the Wassermann reaction, is "flocculated" by the syphilitic antibody. The hog serum, in tending to counteract this flocculation, loses a proportionate amount of its hemolytic action upon sheep erythrocytes. Upon close examination it appears that this reaction is very similar to the other serologic reactions for syphilis. Hitherto it has not been customary to emphasize the colloidal nature of the antigens. But since it has been shown that these antigens are not true antigens, in the strict serologic sense,

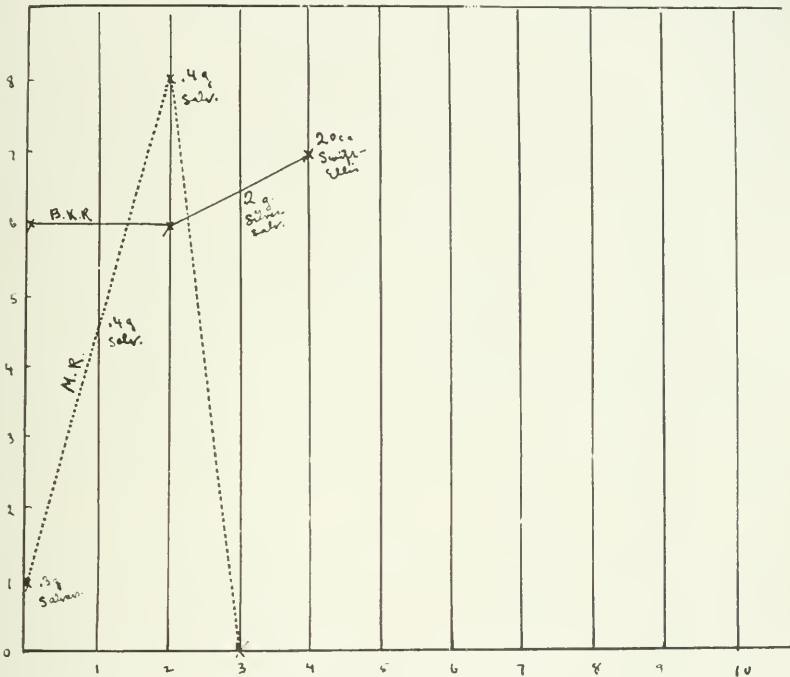


Fig. 3.—Graphic representative of syphilimetric indices in cases under treatment. Initials refer to Table X.

in that they are not specific in relation to the syphilitic antibody, a possibility is presented for considering them from a different aspect, that is, to consider an antigen only in its capacity as an organic colloidal suspension.

The hemolytic activity of the hog serum upon sheep erythrocytes is explained by the presence of native complement and antishoop amboceptor in the serum of most hogs. It is the use of this hog serum which forms the chief difference between Vernes' reaction and the Wassermann.

For practical purposes the use of hog serum presents certain difficulties. In most hospitals hogs are not readily available, and, even if hog serum is obtained, there are instances when it does not contain sufficient complement and amboceptor. In view of these facts it was considered worth while to devise a means of utilizing the colorimetric features of the method of Vernes in conjunction

with a modification of the Wassermann reaction, using guinea-pig complement and rabbit amboceptor, and making special efforts to bring the procedure to a standard basis where consistent results could be secured.

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## DEVELOPMENT OF THE CHEMOTHERAPY OF ORGANIC ARSENICALS AND THE RELATED PHYSICAL PHENOMENA\*

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THE study of the relation of chemical constitution and physiological action has led to an intensive investigation of many chemical substances and has opened up a field of work known as chemotherapy. The specific action of quinine and mercury compounds has long been known. This suggested a careful investigation of the modifications in the constitution of various compounds in which structural changes are made. The work of Ehrlich with metals and dyes, of Einhorn and his coworkers on local anesthetics, Knorr on alkaloids, of Crum Brown and Fraser, and many others on practical therapy, has produced the necessary stimulus for more extensive studies along the line of synthetic chemical research. During the past three decades wonderful advances in the production of antipyretics, analgesics, narcotics and organic heavy metal compounds have been made. Paul Ehrlich has generally been regarded as the father of chemotherapy which is based upon the principle that the selective action of a compound for certain cells depends on the coming together of particular groups in the molecule in some sort of chemical connection with the cell substance. According to this theory it is only when the compound is held to the tissues (anchored) by these groups, that the whole complex molecule can take effect, and exert its characteristic physiologic action.

Witt's theory of dyeing is based upon the condition that the color of a substance is due to the presence of certain "chromophore" groups, such as the azo group,  $-N=N-$ , while for the colored substance to have tinctorial properties, it is necessary for another, salt-forming group to be present by which it can be held fast to the fibre. A dye, therefore, must contain both a chromophore and a salt-forming group, and in the same way a drug must contain an active group, a pharmacophore group and an "anchoring" group or salt-forming group. The analogy may be extended and the phenomena observed in the staining of nerve tissues which is comparable to the biological processes taking place between poisons and living tissues.

According to Ehrlich the process of dyeing is similar to drug action in that the tissues withdraw the active substance from the original solution. With these ideas in mind Ehrlich began the study of organic arsenicals and dyes in relation to curing diseases. The etiotropic action of quinine in malaria has shown that, in those pathogenic organisms which belong to the class of protozoa, the susceptibility toward specific poisons can be greater than that of the cells of the higher organisms, and that consequently such a specific drug

\*Submitted for publication, May 15, 1921.

may produce an internal disinfection without working injury to the host. Particularly useful in enlarging our knowledge of the specific etiotropic relations of this class of pathogenic organisms has been the study of the experimental chemotherapy of the various trypanosome diseases. In 1902 Laveran and Mesnil (*Annal de l'Institut Pasteur*, 1902, page 792), found that these organisms disappeared from the blood of a mouse after the subcutaneous injections of 0.1 mg. of arsenic trioxide per 20 grams of animal but reappeared without repeated administration. However, the mice finally died as the result of the repeated administration of this drug, the curative agent being too toxic to the host in comparison with its efficiency against pathogenic organisms.

These conditions led to further development of etiotropic arsenic therapy which as its culminating achievement produced a curative agent for syphilis introduced as salvarsan. This development was based upon established chemical laws. The pharmacodynamic effects, the laws of mass action, concentration and molecular constitution were all studied in order to find the most satisfactory drug.

The theory of the action of poisons advanced by Loew, furnished a more chemical point of view for attacking the synthetic investigation. This theory states that all substances which are capable of acting on aldehyde or amino groups, even when in dilute solution, must be poisons for living tissue, on which they will exert a substituting action. The greater the reactivity of a substance for aldehyde or amino groups, the greater will be its physiological effect and toxicity. Substances containing tertiary combined nitrogen are usually less toxic than the corresponding substance where the nitrogen is present in the more reactive form of a secondary imino group, exemplified in the case of alkaloids in which there is an alkyl substitution, the resulting tertiary base being less poisonous. In other words, the protoplasmic activity is increased. This view is supported by the fact that the toxicity of substances with labile amino groups is increased by the addition of a second amino group, but is lessened when an amino group is changed into an imino group.

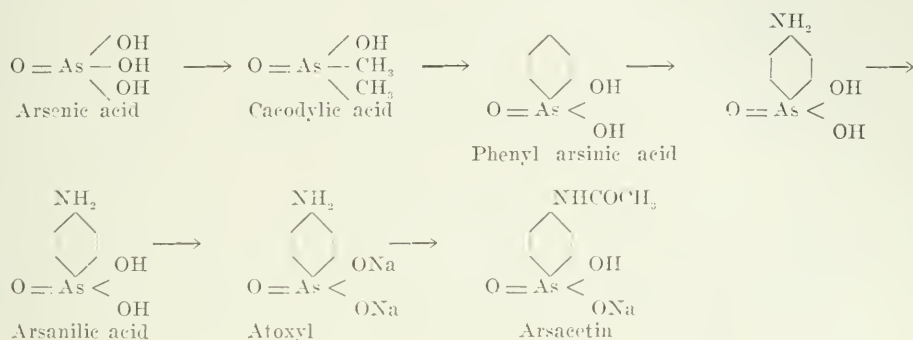
With these theories available, the study of arsenic therapy was carried out. The organic compounds of arsenic contain the metal in the nonionic form, which allows the element to be gradually liberated in the organism, producing the ordinary arsenic effects. However, these are slightly different because the whole organic molecule may alter the penetrative and selective action. Generally, the organic arsenic compounds are more toxic to protozoan parasites and less toxic to mammals. In this way protozoan infections are cured.

In the study on test tube cultures, certain organic arsenic compounds were found to be practically ineffective, but showed some activity in the body. The points at which organic compounds act and, at the same time, the nature of their effects and the order in which they appear, depend on the *physical chemical* properties of the substances in question, these determining whether the complex compounds can penetrate into various organs and cells of the body, to which the simple ionizable metallic compounds penetrate either not at all or only in the course of very chronic poisoning.

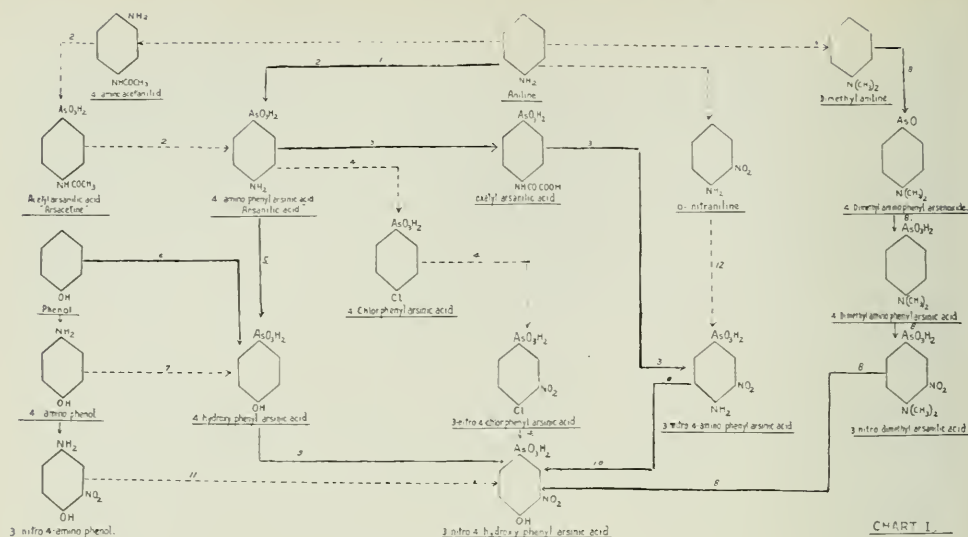
Caedylie acid was one of the first organic arsenic preparations used in

therapy. This, however, is broken up with too great difficulty and consequently is not well adapted for the production of the etiotropic actions of arsenic. This was the real stimulus in a search for organic arsenical compounds which were sufficiently nontoxic and which could be absorbed and carried about in the organism in unaltered form so that they might penetrate into the parasites.

The following structural formulas show the transformations in organic arsenic research.



By means of biological studies it was found that in vitro these substances were relatively inactive but when introduced into the organism it was found that they would kill trypanosomes. However the ratio between the curative dose and the tolerated dose was a small fraction. In order to produce sterilization it was necessary to introduce large repeated doses. This kind of administration produced disturbances throughout the whole organism. These toxic effects consisted of disturbances of the digestive system, and nephritis, unpreventable progressive impairment of vision, and permanent blindness due to optic atrophy. This type of organic arsenic compounds contains arsenic in the *pentavalent* condition and by experiment it was found that they underwent chemical change in the organism to simple *trivalent* arsenic. The toxicity and the disturbances produced by these compounds indicated that further study was required. The fact that these preparations underwent transformation in the organism also suggested the necessity for chemical modification of the molecule. The preparations represented above all contain arsenic in the *pentavalent* condition and notwithstanding the fact that they were supposed to be nontoxic, experience has shown that they are not well suited for therapy. It has been necessary to administer them in relatively large doses and these are excreted so rapidly and in such large amounts that the harmful effects outweigh their efficiency. By introducing repeated large doses, much arsenic is stored up in the body and as a result the kidneys, liver, and tissues are overladen with arsenic. Recently some effort has been made to introduce new pentavalent compounds into therapy but in the light of our present knowledge, modification in the amino group will not offset the bad effects of the pentavalent form of arsenic. Arsenic in the pentavalent form is excreted promptly by the urine except that which is reduced by the organism to trivalent arsenic and



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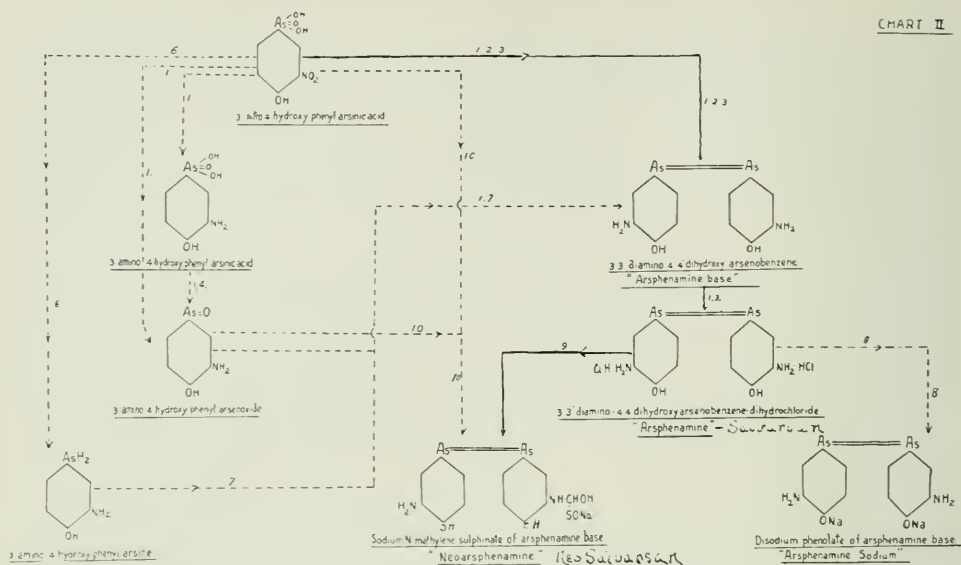
then retained in combination with the body. The kidneys and liver retain large amounts of arsenic and for that reason it is desirable to use a drug which will be as effective as possible in small doses and do the least injury to the body. Fall in blood pressure, renal irritation, anatomic parenchymatous lesions, and nervous phenomena are manifestations following the use of pentavalent arsenic compounds. Blindness and acute arsenical poisoning also result.

The observation that atoxyl did not act upon trypanosomes *in vitro* but was highly active *in vivo* led to the modification of the arsenic molecule. It had been found by Binz and Schultz (Arch. f. exp. Path. u. Pharm., 1879, Vol. 11, page 200) that arsenic pentoxide is in part reduced in the organism to arsenious acid and Ehrlich had prepared p-amino-phenyl arsenoxyd from atoxyl, finding that it was more toxic to trypanosomes when administered in smaller doses.

Although from the above facts it appears that all compounds of *trivalent* arsenic are much more toxic for higher organisms than the pentavalent form, yet it is possible to introduce side chains into the compounds of trivalent arsenic preparations so as to diminish their toxicity. By introducing hydroxyl and amino groups and making the theoretical isomers, it has been possible to select a product which will possess a satisfactory ratio between the etiotropic efficiency and the toxicity. The combination of trivalent arsenic by molecular rearrangement was necessary to obtain this result. The compounds of trivalent arsenic which have met with success in this respect are arseno phenyl glycin ("418"), salvarsan ("606"), neosalvarsan ("914"), salvarsan sodium, galyl (1116), luargol (102), ludyI (1151).

In preparing salvarsan six hundred and six products were made before a satisfactory product was produced. Salvarsan, sometimes called "606" and officially named arsphenamine in this country has been the subject of considerable study from various sources. Much speculation about the practical use of the patents was offered, but it may be said that all of the patents are usable if operated properly. Academic and industrial chemists have collaborated in the work of making and improving arsphenamine and already the drug is being made extensively in France, England, Japan and America. In America the drug is made on a large scale where 15000 grams per day can be made at one plant. The following charts illustrate the methods of making "nitro oxy" the mother substance of salvarsan, and the further development of salvarsan, neosalvarsan, and salvarsan sodium.

It is true that many of these methods are not practical but it has been necessary to study these details in order to provide arsphenamine at a price the most reasonable that has been known. This economic feature is one of the triumphs of large scale production. There are really only three methods in actual use and these are illustrated in the heavier type in the chart. It is necessary to purify all of the intermediates in order to obtain a pure final product. Kober (1919) believed that he was securing a pure salvarsan but failed principally because of the lack of purity of his intermediates. The pure 3-nitro 4-hydroxy-phenyl arsinic acid is a product that exists in two crystalline mod-



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ifications. One form is very pale yellow, and the second is a yellow to red compound.

The second most important step in the preparation of this specific is in the reduction of the pentavalent compound to the corresponding arseno form. This may be done by means of the progressive method in which sulfurous acid with potassium iodide as a catalyst is used, or phenyl hydrazine, thionylchloride, ferrous salts, hypophosphorous acid, phosphorous acid and a variety of other substances may be used. In the reduction of both nitro and arseno groups the catalytic agent, hydriodic acid is very noteworthy and possesses great advantage in activating other reducing substances. By a judicious selection of the reducing agent, the reduction of either nitro group or arsenical radical can be effected at will, more drastic treatment, by means of sodium hydrosulphate, leading to the simultaneous reduction of both these oxygenated complexes.

At this point it might be interesting to give some figures showing the results of the change of arsenic from the pentavalent form to that of the trivalent condition. (Fraenkel, p. 668-1912.) All of these aminoaryl derivatives



are of the greatest therapeutic interest. In vitro atoxyl in 1 per cent solution did not kill a certain strain of trypanosomes, which, however, were destroyed by p-amino-phenylarsenious oxide in dilutions of 1 in 1,000,000. Mice afflicted with very virulent trypanosomes (*nagano ferox*) were healed with atoxyl (1 in 300) only in 5-6 per cent of the cases studied; all cases were healed with p-arseno-phenyl glycine (1 in 600) and this arsenical drug kills in the organism parasites which are immune to atoxyl.

The arsphenamine base is converted into the dihydrochloride using considerable precaution against oxidation. The dihydrochloride is precipitated by anhydrous ether and dried in vacuum. Concentrated hydrochloric acid which is a patented process could be used for precipitation but the physical properties of the final product are materially altered.

Salvarsan is a yellow, amorphous powder whose color varies slightly, due to physical factors unknown at present. However, there is no definite relation between color, toxicity and arsenic content. This salt is readily soluble in cold water, methyl alcohol, and ethylene glycol. Any product which does not possess these properties is not pure in the chemical and physical meaning, nor is it strictly speaking in the chemical sense salvarsan. Contrary to the opinion of Rieger and Kober, arsphenamine contains not only *methyl alcohol*, but also *water*, and *ether*, free hydrochloric acid, together with occasional extraneous inert impurities. For convenience, these amounts of solvents have been expressed as water  $2H_2O$ , but in no case has there been any assertion that it actually was water of crystallization. The original states that this volatile material corresponds to  $2H_2O$ . It has been expressed as water for the very good reason that water is the most abundant volatile product present. Alcohol of crystallization does not exist in arsphenamine prepared in the above manner. To test this point 400 grams of salvarsan were used and if the views of Kober and Rieger were correct there should be about 30 grams of methyl alcohol liberated but the facts were that only a trace of methyl alcohol was found (less than 1 cubic centimeter). The arsphenamine analyzed 31.50 per cent arsenic at the beginning of the experiment with a toxicity of 140 mg. per kilogram of body weight of rat. By proper treatment the sample was converted into anhydrous salvarsan, having an arsenic content of 34.2 per cent with no change in toxicity. This corresponds to theory for arsenic in anhydrous arsphenamine. The experiment showed that there had been no decomposition in the product as illustrated by the toxicity, the physical and chemical properties. The volatile material corresponds nearly to the theoretical loss and consisted principally of water, hydrochloric acid, with traces of ether, and methyl alcohol, the theoretic loss being about 7.5 per cent. This experiment was repeated several times on salvarsan with practically the same results, and also upon other salvarsan substitutes. Salvarsan uniformly possesses a high arsenic content and is readily converted into the anhydrous variety. In some samples of arsphenamine, a maximum of 2 per cent methyl alcohol has been found. The aqueous solution of arsphenamine has a hydrogen-ion concentration of about 4.8 on the Sørensen scale. Two molecules of sodium hydroxide precipitate the free base; the third molecule forms the

mono sodium salt which is soluble in water. The addition of a fourth molecule of sodium hydroxide forms the disodium salt which is the best form for intravenous administration according to the present technic. The hydrogen-ion concentration of the disodium salt is 9.4 on the same scale. The monosodium phenolate is very unstable, easily precipitated by carbon dioxide, phosphates and blood proteins. The disodium salt is comparatively stable. Arsphenamine is precipitated by strong mineral acids and has an arsenic content of about 31.5 per cent, in its commercial form. The Sörenson method does not give absolute figures.

The toxicity tests should be carried out on healthy rats which are free from nutritional disturbances. Toxicity and diet are closely associated, a fact well established by the biological tests of the arsphenamine group carried out by Professor Gies of Columbia University. Unfortunately the biological testing is very far removed from an accurate procedure. The present product can be manufactured on the large scale so that it will uniformly pass a test of 130 mg. per kilogram of body weight. The question of the true nature of the undue toxicity and impurities is still an open one. A few years ago Schamberg, Kolmer and Raiziss (1917) following the lead of Fraenkel in *Die Arzneimittel Synthese*, page 667, 1912, made a number of speculations about the toxic impurities. Through quotation and acquiescence therein many attribute powerful toxic effects to "arsenoxide" which is said to be twenty times as toxic as salvarsan. This product has been eliminated in several ways as an important source of trouble. First, the researches of Hata in "*Die Chemotherapie der Spirillosen*" point out that it is six or seven times as toxic as salvarsan but also that it is twice as active therapeutically as salvarsan. Secondly, the author has examined all the products of all manufacturers for "arsenoxide" over a period of one year and *there is no relation whatever between toxicity and "arsenoxide."* Thirdly, pure "arsenoxide" was prepared and tested biologically as shown in the following table:

DOSE IN MG. PER KILO	NUMBER OF ANIMALS USED	LIVED	DIED
10	5	5	0
15	5	3	2
20	5	2	3
30	5	1	4
40	5	0	5

It thus appears that "arsenoxide" is six or eight times as toxic as arsphenamine but on the other hand according to Hata its therapeutic activity is twice as great. It is very evident that this product is not the primary cause of untoward symptoms.

Substance X suggested by Schamberg et al is readily resolved into three factors. The first is an inherent, known factor of toxicity due to the drug and the second is faulty technic in administration, and thirdly, the physical condition of the product and the solutions. Experiments of the author have shown this last factor to be the predominating difference found in products of any one manufacture. Arsphenamine should be completely dissolved in cold water (20°-30° C.) but we find many references to the use of hot water by

well informed investigators. The acid solution should then be treated with four molecules of sodium hydroxide for each molecule of arsphenamine. This technic was advocated by Ehrlich in 1912 and all literature with the packages called for that quantity of alkali. The use of too concentrated solutions and the rate of injection are the other factors involved in substance X, but are controlled entirely by the predominating factor mentioned above, a specific physical chemical problem. Impurities from the hydrosulphite creep into the final product due to faulty technic on the part of the production chemist and these partly account for the low arsenic content of some products. Therapeutic efficiency is based upon the action of arsenic and it is easily conceivable that products low in arsenic are relatively lower in therapeutic efficiency. These same products cannot be converted into anhydrous products containing 34.20 per cent arsenic on account of the presence of the inorganic inert material.

The presence of sulphur compounds is easily demonstrated, and described by Myers and Dumez (U. S. A. Public Health Reports, 1918, xxxiii, 1003), in which they point out that it is in combined form. Strzyzowski (Zentr. Biochem.-Biophys., 1918, xix, 794) believed that he had found a sulphoacid in arsphenamine. However, at this point there should be a reference to the sulphur compounds described in the German patent, D. R. P. No. 205617, issued in 1907, in which these sulphur derivatives are very carefully described. These so-called new substances were patented and credit given for information to C. Schulte (1882, Ber. d. deutsch. chem. Gesellsch. xv, 1955) and A. Michaelis u H. Loesuer, (Ber. d. deutsch. chem. Gesellsch., 1894, xxvii, 263). Further discussion on this subject is found in the Report by the British Medical Research Committee by Pyman and Fargher (1919). These sulphur compounds are soluble in alkali, precipitated by acids, little soluble in the alkali carbonates, alcohol, and ether. They are said to be more toxic than arsphenamine but possess a correspondingly increased trypanocidal action.

The presence of tetra-amino-dioxy arsenobenzene may be a small impurity when arsphenamine is manufactured by some methods. However, this possible impurity is eliminated when the well-known laws of steric hindrance are employed.

The presence of inorganic arsenic has occasionally been reported in the literature but it is in such small quantities that it is a negligible factor. (For references see Myers and Dumez, U. S. Public Health Report, 1918, Reprint No. 472, p. 5.)

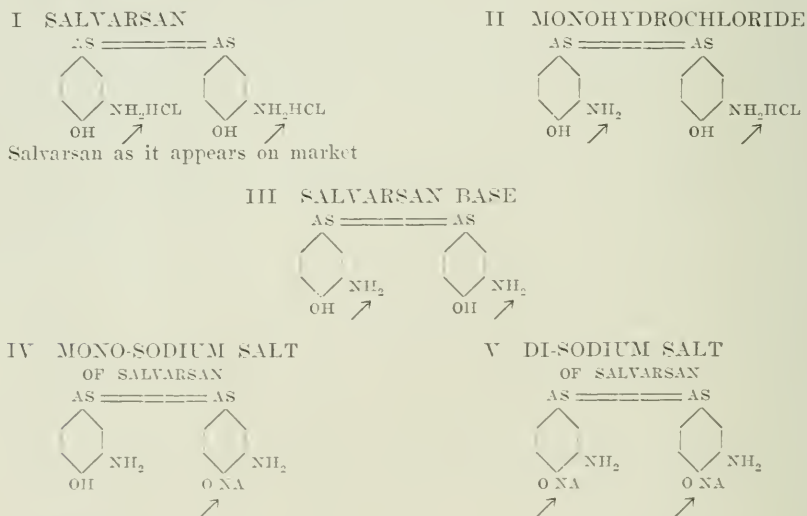
The presence of methyl alcohol can have little to do with the toxicity inasmuch as it occurs in such small quantities. In fact injections of salvarsan in the early days were carried out with the addition of methyl alcohol in order to aid solution. The procedures employed in manufacture exclude the possibility of the formation of the methylated arsphenamines which have been tested out quite extensively.

Excess hydrochloric acid, ether, acetone and water are found when the products are converted into the anhydrous variety. These products will be discussed in another article.

The inorganic impurities are either sulphites or sulphates with a small amount of sodium chloride.

If the question of toxicity is investigated from a more critical point of view, no evidence is at hand to justify a conclusion that individual samples of a given manufacture or even of different manufacture contain substances sufficiently toxic to explain the ordinary variations. The intermediate products and by-products have been quite thoroughly studied and it might be safely said that none of these products do occur in sufficient quantity to offer any explanation of undue toxicity. It appears that the relative "undue toxicity" has been greatly exaggerated due to definite errors of technic explained on a physical basis.

The variations in toxicity have sufficient explanation in small differences in the state of physical dispersion of the product. This is well substantiated by discussion which is to follow. It must be realized that arsphenamine is a semicolloid in the form of the dihydrochloride and during alkalinization it passes through the following stages:



A few test tube experiments will readily show these changes in passing from a relatively disperse phase through a distinct colloid into a second disperse phase as illustrated in the formation of the disodium salt. An exaggerated but often performed clinical operation is worthy of mention at this point showing the effect of physical condition. Many fatalities have been recorded in which the dihydrochloride in water has been injected through carelessness or oversight. The work of Danysz, and Fleig has shown that the high toxicity of this acid solution is not due to its acidity as such. After examining many samples of the commercial product, the author has found that there is about two-tenths of one per cent of free hydrochloric acid present in addition to that which is combined with the two amino groups. It is therefore impossible to attribute variations in toxicity to varying amounts of free hydrochloric acid. In unpublished data the author has isolated the precipi-

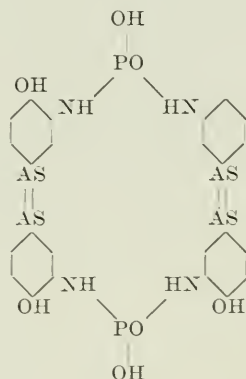


tates formed when the acid solution is added to the blood, serum, and plasma. The amount of precipitate is proportional to the hydrogen-ion concentration of the solution. The largest amount of precipitate is given by the solution of the dihydrochloride, less with the monosodium salt and none with the disodium salt, *when it is added slowly*, or in other words there is a coarser state of dispersion in the case of a solution of the dihydrochloride than in the case of the disodium phenolate solution. When the disodium salt is injected into the blood stream, the high hydroxyl-ion concentration tends to allow a finer state of dispersion and also permits a more uniform rate of chemical transformation. In fact these blood precipitates are so characteristic for different products that one can identify the product by the nature of the precipitate. In addition to the physical changes taking place in the drug itself, account must be made of the modifications of the physical state of the blood stream itself. Calcium unites very readily with arsphenamine and at certain  $p_H$  values is a definite yellow precipitate. The same applies to magnesium, therefore it is advisable to keep the solution definitely on the alkaline side of the neutral point.

“Colonel Harrison has reported to the Committee the curious behavior of certain samples of ‘606’ which, though apparently not inherently toxic to an abnormal degree, since they may be given with impunity if carefully made up with freshly distilled water, readily cause symptoms of vasomotor disturbance if made up with boiled and filtered tap-water. In contrast to these ‘sensitive’ samples, he finds that other batches of the product never cause abnormal reactions, whether made up with boiled tap-water or distilled water. It would be very difficult to reconcile such an experience with the view that the abnormal toxicity depends on the presence of an undue proportion of more toxic by-products. It is more readily intelligible, though by no means susceptible as yet of detailed explanation, on the view that the variations of toxicity are due to small differences in the physical properties of different samples of the preparation.

“This view, as to the relation between the toxicity of ‘606’ and the state of dispersion in which it separates from solution on neutralization by the blood, has been given more definite form by some French observers. The alkaline solution, when exposed to air, sooner or later shows an incipient turbidity, as the alkalinity becomes reduced by absorption of carbon dioxide. It has been stated that samples, the alkaline solution of which begins to become cloudy and to give a deposit within 10 minutes, will readily cause vasomotor disturbances (‘crises nitritoides’) on injection. This statement does not accord with the evidence obtained from the official tests of ‘606’ in this country, the results of which have been put before the Committee. Many samples showing this early tendency to separation of the base have been perfectly satisfactory from the point of view of toxicity, whereas others, the alkaline solution of which remained perfectly clear for a long time after preparation, have exhibited an acutely toxic action leading to their rejection.” (British Med. Res. Committee, 1919.)

Our usual chemical analysis has led us to assign the formula  $R\ As = As\ R$  to salvarsan but more detailed analyses from the physical chemical point of view will probably point to a greater association of molecules than that which is illustrated by the usual formula. In this connection it is interesting to note the structural formula which is ascribed to Galyl, a phosphoric acid derivative of arseno-benzol (so called by the French). The formula for this product is as follows:



The freezing point depressions which have been carried out in this laboratory show that the lowering is extremely small, indicating that there is either a distinct colloidal condition or a relatively large association of molecule, a phenomenon similar to the observations of Morse and Myers in their studies on osmosis at  $0^{\circ}\text{C}$ . in the dilute sugar concentrations. (Carnegie Institute for Advancement of Science—Monograph on Osmotic Pressure.)

In relation to these physical differences recently observed we should not fail to refer to a statement by Ehrlich and Bertheim (Ber. d. deutsch. chem. Gesellsch., 1912, xlv, 764). The amount of alkali used in various preparations, the kind of solvent whether it is *pure* water, water *containing alcohol*, *physiological saline*, and the rapidity by which the alkali is added play an important part in the solution of salvarsan. Small deviations make great variation in the solution and it seems probable that colloids enter into these changes.

On this basis this laboratory has always recommended a uniform procedure in the preparation of solutions of arsphenamine. The author has conclusively shown that reactions could be almost entirely eliminated by this procedure. The product is dissolved in a definite number of cubic centimeters of water and then exactly and uniformly alkalinized. Overemphasis on the matter of shaking has appeared in the literature but it should be pointed out that shaking *acid solutions* causes no harm, and at *low temperatures* little trouble will be experienced with alkaline solutions.

Dr. G. C. Lake, formerly of our staff, has definitely shown that there is a decrease in toxicity when salvarsan is allowed to stand thirty minutes or more after the addition of the alkali. The alkali should be added all at once.



MG. OF DRUG PER KG. OF ANIMAL	NUMBER OF RATS	TIME OF STANDING	SURVIVALS
100	5	Immediate	0
120	5	"	1
130	5	"	0
140	5	"	0
120	5	30 min.	5
130	5	30 min.	5
140	5	30 min.	3

These tests were carried out on an average grade of rats obtained from the open market. By regulating the diet as pointed out by Prof. Gies, the toxicity figures would be materially lowered. On a well regulated diet the toxicity is from 20 to 30 mg. lower. In unpublished data, Hooper has shown that 60 per cent of the animals will survive at even higher doses when the rats are subject to quarantine and given a prescribed diet as given in detail in his article. In the above table the changes in toxicity are quite manifest as the time of standing is increased. If the formula for colloids is recalled the factors, time and temperature are observed to be extremely important. The application of time, temperature, and concentration are insufficiently emphasized in the matter of testing arsphenamine and also in its clinical use.

In relation to the colloidal properties of the arsenicals of the "arseno" type, the work of H. Bauer (Arbeiten aus dem Inst. f. Exp. Ther. u. dem, Georg. Speyer-Hause, 1919, No. 8, page 45) is of special interest. These experiments were carried out in the course of their investigation on the physical chemical conditions of aqueous solutions of these complex arsenicals. These investigations are extremely important in the clinical use of these products which show such a marked tendency to separate out, to dissolve and to gelatinize. Special interest is attached to the addition of one molecule of silver nitrate to one molecule of salvarsan. At first there is an *orange* colored solution formed; then if alkali is added the free silver salvarsan base is precipitated as a dark *yellow* solid dissolving in an excess of alkali producing an intensive dark *brown* solution (change in color). Observations of this kind induced Bauer to look for colloidal changes. He used the methods of Graham. (Phil. Trans., 1861, p. 183), Biltz (Ztschr. f. physikal. Chem., 1910, lxviii, p. 365), H. Bechold (Die Kolloide in Biologie und Med. 2nd edition, p. 114). By these methods he was able to study the size of the arseno particles and their diffusibility through the ultrafilter. A color change similar in nature is observed during the transformations described on page 26 of this article.

During the course of Bauer's investigations it was found that a large part of the dihydrochloride solutions was held back by the ultrafilter, showing that there were colloidal particles of large diameter remaining in the solution, while the dry sodium salt when dissolved showed less colloidal tendency than the freshly prepared disodium salt.

The arseno compounds have been arranged by Bauer in this order:

1. Collargol (Colloid)
2. Salvarsan Silver Sodium
3. Neosalvarsan
4. Salvarsan dihydrochloride

5. Salvarsan freshly alkalized
6. Salvarsan Sodium
7. Silver nitrate (krystalloid)

The conclusion drawn from these experiments is that these products are semicolloids easily changing their physical condition under the influence of other physical factors.

The last point to be discussed in this paper is the question of viscosity, a physical factor of much importance in the study of matter in solution. Shernadal in a paper (unpublished) read before the American Chemical Society in Rochester, 1921, has pointed out some very significant facts dealing with the changes of the viscosity of products in solution and their relation to the nature of the precipitating media. The theoretical nature of viscosity of solutions and colloidal solutions is discussed at length in numerous treatises on this subject. The report of Z. Klemeniewicz (Sur les proprietes colloïdales des solutions aqueuses du Salvarsan (Bulletin de la Societe Chimique de France. Vol. 27, pp. 820-824): "The dichlorhydrate of dioxy-4, 4'-diamino-3, 3'-arsenobenzol, better known under the name of 'Salvarsan' or product '606,' is a substance which presents a decided interest not only for medicine, but also for pure chemistry. The facility with which its molecules react with the most varied substances is known. It would not astonish us then to learn that *they also unite among themselves to form large or complex molecules.* In preparing slightly concentrated solutions of Salvarsan, it is seen that upon contact with its derivative, sulphoxylate (neosalvarsan or '914'), 606, instead of dissolving, *at first absorbs water, forming a jelly* which is only slowly dissolved. It is striking that it is especially the last remains of the jelly that take the longest time to dissolve, although the solution is far from saturated. In looking for an explanation of these phenomena, I undertook the study of the viscosity of solutions of 606 by the ordinary method of flowing through capillary tubes. My experiments showed that the viscosity of a given solution is not immediately constant, but grows from the moment of preparation to attain an approximately constant value, much higher than the initial value.

CONCENTRATION	I $N \propto$	II $\frac{d\eta}{dt}$
2 %.....	2.3	0,00015
4 .....	3.7	0,00083
6 .....	8	0,0039
8 .....	20	0,012
10 .....	36	0,030
12 .....	140	0,13

"The full curves of the chart illustrate the change of the relative viscosity (the viscosity of water at 15° being chosen as unity) of solutions of 2, 4, 6, 8, 10 and 12 per cent, maintained at a temperature of 15°. The abscissæ mark the time in minutes. Tables I and II contain respectively the first values of viscosity and the initial speed of increase  $\frac{d\eta}{dt}$  .

"1st. It is seen that the initial speed as well as the final value increases very considerably with the concentration of the solutions. This explains the fact that concentrated solutions of 606 cannot be obtained practically, because they gelatinize after a time, the more quickly in proportion as they are more concentrated. Another disagreeable consequence is observed through the exactitude of our measurements. Already during the preparation of the solution its viscosity increases in a variable manner and the beginning of the time is consequently badly determined.

"If by diluting a 10 per cent solution which has already attained its final value, *less concentrated* solutions are *quickly* prepared, these show values of viscosity *higher* than the corresponding  $\eta\alpha$ . These values decrease with the time so as to approach sufficiently the values  $\eta\alpha$  of this table. The least concentrated solutions are those which best illustrate this phenomenon.

"2nd. If we maintain the solution between two measurements at a *higher temperature* (the measurements themselves are always made at  $15^\circ$  and several minutes are allowed to elapse so that the solution and the apparatus take this temperature) the viscosity more quickly attains its  $\eta\alpha$  but this value is smaller than for a solution maintained at  $15^\circ$ . The broken curve refers to an experiment made at  $25^\circ$  with a solution of 12 per cent. A 10 per cent solution, sealed in an ampule filled with nitrogen, was heated for 5 hours at  $50^\circ$  and then left for 4 days at  $15^\circ$ . It only attained  $= 15$ , or less than half or the corresponding  $\alpha$  (36). It is observed that complete reversibility is lacking, which may be due to a chemical composition.

"3rd. The titer of acidity influences the turn of the curves. The dotted curve shows the variation of the viscosity of an 8 per cent solution in normal hydrochloric acid. The increase of  $\frac{d\eta}{dt}$  is observed, the value of  $\eta\alpha$  is tripled. The viscosity then decreases, which is due to a chemical action of the acid, for a precipitate soon appears. Since the trade product shows very noticeable differences in titers of acidity\* the entire series of measures must be made on the samples of the same lot, which has been the case in the present study. Our product contained 0.142 gr. HCl per gram of salvarsan. I believe, furthermore, that the *degree of polymerisation* (see below) of each lot is *influenced by its past*, especially *since the change continues in the solid state*. A solution of 10 per cent having attained its  $\eta\alpha$  was precipitated and redissolved with a few drops of concentrated caustic soda. The viscosity fell suddenly to 2.1, and the fall was prolonged for two days attaining 1.3, which represents approximately the viscosity of a 10 per cent solution of product 914. Also the viscosity is in order of size, when pertaining to solutions of sodium salts of cinnamic and phthalic acid and of other aromatic substances of a similar molecular size.

"According to this result, the aqueous acid solutions of 606 are ranged among the colloids called stable 'lyophiles' or 'emulsoids,' with solutions of gelatine, albumen, etc. It must be noted that product 606 possesses likewise the (aminee) function. As its structure is known, the study of its properties

\*The theoretical acidity of dichlorhydrate is not maintained in practice.

could perhaps assist as in the investigation of the above mentioned substances. It is besides not without interest for the exploration of the mechanics of therapeutic action of 606.

“As regards the cause of the phenomenon described here, it could straightway be supposed, in view of the great power of 606 of being oxidized that the increase of viscosity is due to a chemical reaction of this kind. But it is found that the phenomenon is perceptibly the same for solutions maintained in an inert gas, and that in all cases the free surface was small. Otherwise the oxidation of 606 produces known substances, well crystallized, the solutions of which show a normal viscosity. We must, on the contrary, believe that

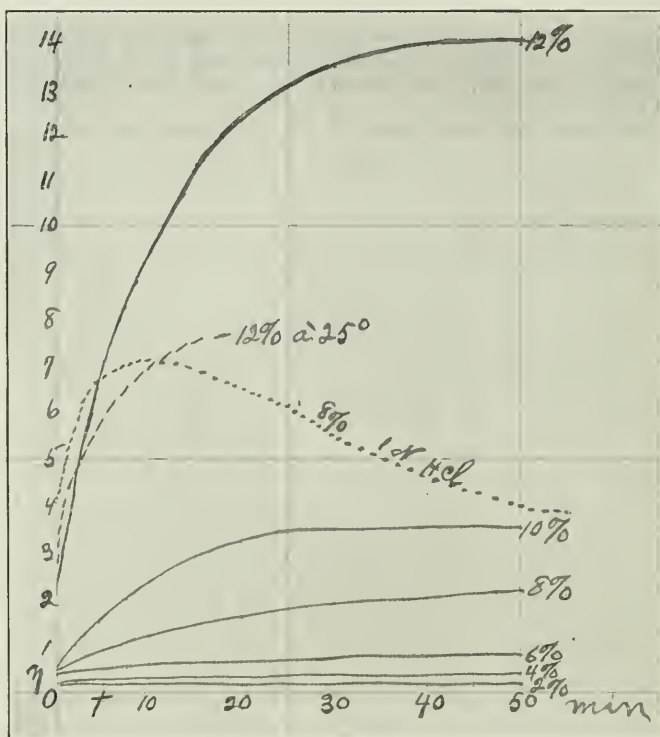


Chart 111

solutions of salvarsan give rise to very strong polymerisation of the molecule, probably accompanied by hydration. This reaction seems to obey the general laws of chemical mechanics, as the action of the temperature and of the concentration show. It is interesting that it may be due to ions  $(\text{AsC}_6\text{H}_3\text{OHNH}_2\text{H})^+$ , while ions  $(\text{AsC}_6\text{H}_3\text{NH}_2\text{O})^-$ , present in alkaline solutions, do not seem inclined to this transformation.”

W. L. Miller (Jour. Phys. Chem., 1920, xxiv, 562) has shown that two solutions have the same toxicity only when they are in equilibrium with their constituents in the solutions and have the same *potential*. Complications may arise from the toxicity of the salts themselves or in dilute solutions from the plasmolysis of the cells, independent of the toxicity of the solutions employed.



These facts are of much significance in the explanation of why one clinician gets no reactions while another using identically the same product under slightly different conditions may have some trouble.

#### ADMINISTRATION

From a chemical point of view, the investigator immediately raises the question: Why does Salvarsan react with protozoa and blood proteins? These questions are fundamental to the whole subject of chemical dynamics and chemical equilibrium, which factors are actively and constantly engaged in carrying out the body functions. The cause of all chemical reactions is to be found in the fundamental laws of energy changes. Every energy change can be factored into two parts, an intensity factor and a capacity factor. These last two factors are causes of disturbances in the administration of arsphenamine. It is well known that, when two substances are brought together, there is a tendency to equalize the differences in the intensity of the intrinsic energy producing the chemical change and on this basis the technic of administration must be carried out with the utmost care. A more homely illustration might be used in which a piece of sodium hydroxide is dropped in concentrated sulphuric acid with a violent reaction, whereas if dilute solutions of the same reagents are used, no perceptible change is noted, even though the equilibrium point is the same.

These same laws apply to arsphenamine. Large doses in concentrated solution when injected rapidly produce localized intensified energy changes which finally extend over the whole circulatory system. Whereas, the injection of more dilute solutions allows the gradual equalization in equilibrium. Arsphenamine forms a chemical combination with the protozoa and also with the blood protein and the rate of formation determines the intensity of the reaction. The arsphenamine should be carefully alkalinized with four molecules of sodium hydroxide after the dihydrochloride is *completely* dissolved in cold water. This clear solution should be diluted in the proportion of one decigram to twenty or twenty-five cubic centimeters of freshly distilled water. The intravenous administration should be carried out in about a half hour after the preparation of the solution at a slow rate of flow. With this technic the possibility of the formation of precipitates is almost entirely eliminated.

In this connection it is interesting to call attention to the recent work of Oden, 1920. This work has a direct bearing on the clinical use of the drug, a point long emphasized by the author of this paper. Oden states that precipitates formed in ionic reactions consist of single-grain particles (primary particles). The size (radius,  $r$ ) of the particles is determined by the manner in which the solutions are poured together, temperature, and the concentration of the electrolytes. Primary particles remain in their single-grain structure only in the presence of a definite concentration of electrolytes (critical concentration). The concentration of an electrolyte necessary to maintain the primary particles as such is characteristic of the suspended particle and of the salts in the solution. The critical concentration may on the one hand be very narrow in range (perhaps less than 0.001 mol.) or on the

other hand, have rather wide limits (as in my experiments with  $\text{BaSO}_4$ -potassium citrate, 0.001 to over 1.0 mols.). When the amount of the electrolyte passes the critical concentration, the primary particles unite to form aggregates (secondary particles). The size ( $r$ ) of the secondary particles is dependent upon the electric charge of the primary particle as determined by its radius, also by the concentration of the primary particles and the concentration of the electrolytes in solution. In the majority of precipitates from ionic reactions the radius of the particles attains a fixed maximum, which is determined by the electrolyte concentration. When a precipitate consisting of secondary particles is shaken up in water the particles are resolved into their primary particles. Such primary particles reaggregate when the fluid comes to rest. In re-forming secondary particles these assume a size appropriate to the concentration of electrolytes present, and if this concentration has been changed to a point nearer the critical concentration, the radius of the re-formed particles will be less than of the original secondary particles. By careful adjustment the radius may in this manner be reduced to the radius of the primary particles (disaggregation). Such secondary particles are said to be reversible.

With a decrease in the potential of the primary particle there is an increase in the number of primary particles in the aggregate. The author conceives the potential of the particles as dependent upon the anions and cations adsorbed, and this in turn upon the radius of the particle, also the chemical nature of the particle and the individual ions of the coagulator.

It has been shown in our laboratories that there is a decreased toxicity when the solution is allowed to stand thirty minutes. In many instances the decrease in toxicity amounts to as much as 30 per cent. This peculiar change in toxicity undoubtedly throws a peculiar light upon many reports on the toxicity of the drug where time, temperature and concentration have been dealt with in an inaccurate manner. There is no doubt that there is need for a marked revision of the literature on this subject. The question of standing is readily explained either on the basis of intramolecular rearrangement or on the basis of the change of the physical state of the molecules.

#### CLINICAL FEATURES

After a little consideration it will be observed that the end results are those which are most important. Unfortunately this phase of research does not receive as thorough analysis as it should. Realizing this, the work at our laboratories has been directed to the point of coordination of manufacturing, chemical, biological, and clinical facts. A drug has no practical use unless it can alleviate or perhaps cure a disease when it is administered in the most efficacious manner. In this respect, there is too great a diversity of pseudo-scientific opinion. The specific object sought after in this instance is to cure syphilis and its effects on the large scale, but on account of the unreliability of patients who fail to grasp the seriousness of the disease and the proper approach of the physician, good intentions go wrong. There is a lack of the knowledge of the frequency of treatment, the size of the dose, and the con-



traindications. At present repeated 0.4 gram doses of salvarsan are generally advocated, but the frequency of treatment is a wide variable. The use of repeated fractional doses is a technic that has been advocated as long as the drug has been on the market. There are still a few who adhere to the large (0.6 gram) doses without any scientific reason whatever. The physician has failed to grasp the point that biological processes are governed by the laws of chemical dynamics and that an arsenic equilibrium must be established in the host and in the parasite in order to produce a cure. Investigation is now going on with the intention of establishing this point in its relation to frequency and size of dose with the greatest safeguard to the patient. The frequency of administration and the rate of elimination are the factors necessary to determine this equilibrium. At present we get cures and reduced Wassermann test, but on a scientific basis and by means of biologic work these results should be more frequent. Inasmuch as it is almost obligatory to receive treatment for venereal disease, it seems wise that standard methods should be devised.

#### REACTIONS

Reactions are the phenomena which disturb the physician and the patient and the manufacturer in their relations one to the other. Conscientiously no one desires to be the cause of fatalities and consequently it has been necessary to assist and teach physicians the best way of using a synthetic chemical which unfortunately does not tacitly submit to misuse. Literature provided with these finished products is of little use inasmuch as it readily finds its way to the waste basket. In order to assist in the propaganda against venereal disease it has seemed best to study these questions in the clinic. In 75 per cent of the cases reactions have been due to faulty technic where poor water was used, or under alkalinized solutions, or old solutions were employed in the administration. Also the use of concentrated solutions given rapidly with a syringe should be included in this class. To test out this question thoroughly several hundred injections were made with the same product, using the same technic throughout, with the exception of concentration. In the case of the concentrated solutions, there were more minor disturbances although nothing serious resulted, while in the dilute solutions given slowly by gravity no trouble whatever occurred. W. H. Wilcox (*Lancet*, May 24, 1919, p. 872) in his Lettsomian Lectures points out that arsenobenzol derivatives have a powerful toxic action on the liver cells and an intense degeneration with much fat deposition is found in the liver and kidneys in fatal cases and in consequence of the danger of the occurrence of grave toxemia the dose has been reduced. Other types of reactions that occur are flushing, coughing, diarrhea, pains in the back, and in severe cases vomiting, convulsions and coma may result. Reactions appear to be more frequently set up by the use of the monosodium salt which is readily decomposed in the venous blood yielding precipitates which clog the capillaries. These precipitates have been isolated and analyzed. If the treatment is continued over too long a period the kidneys and liver show signs of chemical poisoning and a rest is desirable. The diet and physical condition of the patient should be carefully

watched in addition to the attention given to blood pressure, examination of the urine, etc. When the body reaches the maximum arsenic equilibrium, it is unsafe to continue the injections.

If we apply some of the physical chemical suggestions discussed in the preceding paragraphs, it is readily observed that they have a direct application to the subject of reactions. Colloids are common to the blood stream in the sense that the surface affinities have already established an equilibrium. Now if a foreign colloid "in solution" is introduced with varying viscosity, potential, and dissociating powers, it is apparent that there is immediate need for the establishing of an equilibrium. Hydrogen ions tend to aggregate (precipitates) as in the case of the acid solutions and the monosodium salt in the presence of carbonates, whereas the hydroxyl ions tend to disperse as in the case of the disodium salt. With these views well substantiated by clinical findings, it readily appears that detail study of the dynamics of these products in their relation to therapy is desirable.

It has long been pointed out that acute reactions are principally disturbances of the physical equilibrium of the blood stream. If injections are made with dilute solutions, well alkalized and injected slowly, a precipitate of very small diameter may be formed which is quickly converted by the body into a soluble and easily utilizable product. This converted protein compound is the one which is deposited in the tissues and by its slow decomposition protozoa are killed. This type of drug might very easily be called the "active" drug, and the one which actually produces the etiotropic effects. These physical phenomena are being studied more in detail to determine the extent and the exact nature of their application to the question of reactions.

In this connection Sicard and Paraf (1921) have aptly suggested: "The 'shock' resulting from the intravenous administration of the novarsenicals or arsenical colloidelasia is attributed to a disturbance of the colloidal state of the body fluids. The authors observed that the injections of sterile solutions of  $\text{Na}_2\text{CO}_3$ , which had been kept in containers of soft glass, were frequently followed by similar phenomena. This they attribute to the products of the reaction of the  $\text{Na}_2\text{CO}_3$  on the glass, since when solutions from hard-glass containers were used no such after-effects were obtained. When 30 c.c. of physiological saline solution containing 0.6 to 0.7 g. of  $\text{NaHCO}_3$  are injected intravenously just before the administration of the novarsenical no untoward results occur." This point was early pointed out by Myers, 1919, 1921, and resulted in the development of the automatic burette for preserving and using alkali.

In order to arrive at definite knowledge about drugs, it is necessary for the closest cooperation to exist between industry and biological and physiological testing. The end of chemotherapy studies is not in sight and it is safe to say that in the near future new and more valuable remedies will be synthesized. This can only be accomplished by the application of known physical and chemical laws to physiological processes. The time has arrived when the study of chemotherapy will be applied in the chemical industry and the future supply of drugs will be readily available.

## SUMMARY

1. The chemotherapeutic development of arsenicals is discussed showing the relation of pentavalent arsenical products to those of the trivalent condition as illustrated in the "arseno" type.

2. The rationale of the physiologic action is discussed from the chemical point of view showing the effect of the chemical action of mass, concentration, and pharmacodynamics.

3. A complete chart showing all the possible methods of arriving at the final product salvarsan (*with references*) is given.

4. Methyl alcohol of crystallization is discussed with sufficient fullness to exclude it in the calculation of the often debated " $2\text{H}_2\text{O}$ " content.

5. Anhydrous arsphenamine is a practical test of the purity of the technical products.

6. The lethal dose of "arsenoxide" is found to be about 25 mg. per kg. of animal weight.

7. Impurities and their relation to toxicity are discussed. There is no relation between "arsenoxide content" and toxicity of various samples.

8. The chemical transformation of the product is shown in a chart showing the opportunities for physical chemical changes to take place.

9. The physical chemistry of arsphenamine is given with reference to the presence of colloidal properties and a discussion of the question of solutions in reference to viscosity, potential, dissociation, equilibrium, and ultrafiltration and their relation to toxicity.

10. The administration of the drug in relation to concentrated and dilute solutions is discussed. Standing for thirty minutes tends to decrease the toxicity of freshly alkalized solutions of arsphenamine.

11. Need of clinical standardization is pointed out.

12. Reactions are explained in terms of disturbances of chemical equilibrium (precipitates).

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# VENTILATION, WEATHER, AND THE COMMON COLD

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## A STUDY OF THE PREVALENCE OF RESPIRATORY AFFECTIONS AMONG SCHOOL CHILDREN AND THEIR ASSOCIATION WITH SCHOOL VENTILATION AND THE SEASONAL CHANGES IN WEATHER

*(Continued from vol. vi, page 698)*

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### DISCUSSION OF VENTILATION TYPES REPRESENTED IN THIS STUDY

The conclusions arrived at in discussing the relation of ventilation to colds raise the question as to whether all methods of school building ventilation by mechanical means are discredited by this test. This inference is of course unwarranted. The results of this study apply only to the types of ventilation here represented, a brief description of which will be given.

The selection of schools was made by the writer after a conference with Mr. Frank G. McCann, heating and ventilating engineer of the New York City Board of Education. Altogether over fifty schools were considered, and more than half of this number were visited in person by the writer.

There were many factors to weigh in making the selection. It was desired above all to choose schools possessing all three types of ventilation. As previously explained, this was found impossible except for one school. These conditions were nearly fulfilled in one other instance where the three types were found in two schools within a block or so of each other. For convenience it was desirable that the schools should be located within Manhattan and Bronx Boros. To represent this territory, selections were made in Lower Manhattan, Central Manhattan and Upper Manhattan and the Bronx. It was further desired that the ventilation methods should have already been in use for some time prior to the experiment, so that uniform operating conditions would be established. It was possible to adhere to this requirement in all but one school, 165, in the first study and all but four in the second. In these four, 165, 97, 115 and 33 Bx., window ventilation was arranged by blocking off the fan supply inlets to the rooms and by installing deflectors at the windows. The effort was made to select schools whose mechanical ventilation equipment was in good condition and in capable hands. Lastly it was essential that the principal of the school should be in sympathy with the objects of the study.

Schools 12, 147 and 22 were located in lower Manhattan, the first on Madison and Jackson Streets; the second on Henry and Gouverneur, and the third on Stanton and Sheriff. Schools 59 and 73 were in Central Manhattan, the former on 57th Street near Third Avenue, the latter on 46th Street near

Third Avenue. School 165 was on 109th Street near Broadway; 39 on 126th street near Second Avenue; 2 Bx. on 169th Street near Third Avenue. The schools added in the second study were 97 on Mangin Street between Stanton and Houston, in the lower East Side; 115 on 176th Street near Audobon Avenue; 33 Bx. on 184th Street and Jerome Avenue, Bronx; 51 Bx. on Trinity and Jackson Avenues, Bronx.

The date of erection of each building, as well as the type of building ventilation is shown in Table XXII:

TABLE XXII

SCHOOL	DATE OF ERECTION AND ADDITIONS	AERATION	HEAT CONTROL	REMARKS
12	1908	Window and Grav. Exhaust	Thermostatic	
147	1898	Plenum Fan and Grav. Exh.	"	
22	1843-1873-1891-1902	" " " " "	"	
73	1880-1902	Windows and Grav. Exhaust	Manual	
59	1871-1904-1908	Plenum Fan and Grav. Exh.	Thermostatic	Also air washer
165	1898-1905	" " " " "	"	
39	1903	Windows and Grav. Exh.	"	
2 Bx.	1874-1886-1902	Plenum Fan and Grav. Exh.	"	
51 Bx.	1915	Plenum and Exhaust Fans*	"	Also air washer*
33 Bx.	1899	Plenum Fan and Grav. Exh.	"	
115	1914	Plenum Fan and Grav. Exh.	"	Also humidifying fan
97	1915	" " " " "	"	Also air washer

\*This refers only to the two rooms used in the study.

The window ventilated rooms had direct radiation beneath the windows. In the first study there were no deflectors except at School 73. In the second study deflectors were provided in all rooms of Type A and B. All of these rooms had gravity exhaust openings to permit air circulation. It is best that these openings be in the inside wall opposite the windows. This arrangement did not exist in many rooms, the openings being in walls adjacent to the windows and frequently so small in size as to be really of little service in keeping the room well aerated. Direct radiation was thermostatically controlled in all cases save in School 73, where it was manually controlled.

The mechanical ventilation consisted usually of plenum fans, air being driven into the classrooms near the ceiling and leaving through gravity exhaust openings near the floor. These rooms were also provided with direct radiation under thermostatic control. A check on the ventilation was had by means of ribbons on the inlet. These were observed by the nurses, who could thus tell in a rough way the efficiency of the blowers.

The system at School 147 gave a weak and irregular air flow to the three classrooms during the first study, and windows were frequently opened. This condition was greatly improved in the second study.

School 22 had a plenum fan system in the new wing. The air flow in the rooms was continuous and pronounced, and windows were rarely opened. Anemometer readings at the register face made on January 8, 1917, showed 1640 cubic feet per minute in Room 402, 1960 in 403 and 1620 in Room 404.

School 59 had a plenum fan system in the new addition built in 1908. This consisted of two blowers and an air washer. This equipment was in most capable



hands, and the rooms were at all times well flushed with humidified and washed air. Windows were always kept closed. On January 15, 1917, anemometer measurements showed the following air flow in cubic feet per minute: Room 203—1400, 205—1300, 206—1740, 501—1150, 503—1380, 505—760. During both studies there was continuous and pronounced flow in all rooms.

School 165 had a plenum fan system with gravity exhaust which proved inadequate for the rooms used in the study. At times air flow was ample, again deficient. Conditions were better in the second study.

School 2 Bx. consisted of an old and new section, the latter built in 1902. This section contained the plenum fan ventilating equipment with gravity exhaust. Air flow was fairly continuous and pronounced.

The orientation of the classrooms varied somewhat in the three types, the A rooms having a greater amount of southern exposure.

TABLE XXIII  
ORIENTATION OF CLASSROOMS IN FIRST STUDY

VENTILATION TYPE	N., N. E., N. W.	W.	S., S. E., S. W.	E.
A	5	0	12	1
B	9	3	4	5
C	9	5	3	2

#### RESULTS IN NEWER SCHOOLS ADDED IN SECOND STUDY

When this study was begun, there were no schools erected within two or three years which could be used. In the second study we were enabled to add four schools, three of which had just been opened to pupils within the year. School 51 Bx. contained two rooms in which the Ventilation Commission was making studies on the effect of humidification, both being ventilated by plenum and exhaust fans. These results were included in the grand totals, but of course they do not furnish us with a comparison of the types forming the basis of this study. The respiratory sickness rates were higher in the humidified room. Temperature was about the same in both. Odor was frequently noticeable in the humidified room.

TABLE XXIV  
RESPIRATORY ILLNESS IN SCHOOL 51

VENTILATION TYPE	ABSENCE RATE	SICKNESS IN AT- TENDANCE RATE	TOTAL RESP. SICKNESS RATE	TEMPERA- TURE	RELATIVE HUMIDITY	PER CENT SESSIONS	
						ESP. FRESH	ODOROUS
Humidified	4.0	148.	152.	67.6	44	0	39
Not Humidified	14.7	50.	64.7	67.4	29	0	2

School 33 was an older building, but because of work already in progress there by the Ventilation Commission, the sickness records were also collected and added to the grand totals of this study. Ventilation was by

means of plenum fans and gravity exhaust. The fan inlets were blocked off in the Type B rooms and deflectors placed at the windows. The respiratory sickness rates were less in the two window ventilated rooms than in the two of Type C. The fan rooms were slightly warmer but less odorous.

TABLE XXV  
RESPIRATORY ILLNESS IN SCHOOL 33

VENTILATION TYPE	ABSENCE RATE	SICKNESS IN AT- TENDANCE RATE	TOTAL RESP. SICKNESS RATE	TEMPERA- TURE	RELATIVE HUMIDITY	PER CENT SESSIONS	
						ESP. FRESH	ODOROUS
B	24.2	52	76.2	69.4	34	1	15
C	37.0	72	109.0	70.6	30	2	3

School 97 was just completed in 1915 and was equipped with plenum fans and gravity exhaust, the air being washed and humidified before entering the rooms. Three rooms were chosen to represent each of Type B and C. The respiratory sickness rates were the lowest here of all the schools. Sickness was less in the fan ventilated rooms than in the window rooms. The fan rooms were a degree warmer and were much better aerated.

TABLE XXVI  
RESPIRATORY ILLNESS IN SCHOOL 97

VENTILATION TYPE	ABSENCE RATE	SICKNESS IN AT- TENDANCE RATE	TOTAL RESP. SICKNESS RATE	TEMPERA- TURE	RELATIVE HUMIDITY	PER CENT SESSIONS	
						ESP. FRESH	ODOROUS
B	5.6	47	52.6	66.7	42	49	32
C	3.7	37	40.7	67.6	43	85	3

The location of this school was along the East River, and across the street was a large stable whose odors were frequently wafted over to the school at times of east wind. This interfered with the free use of the windows. The fan ventilated rooms were not troubled by these odors. One of the three window rooms was on an enclosed court which also cut down free air circulation. The experience at this school was very suggestive. Here was an illustration where fan ventilation provided a more satisfactory result than window ventilation. Schools with similar locations in the neighborhood of noxious odors are evidently better served by indirect washed air than by taking air direct from the windows. The teacher of one window room was well satisfied and much preferred it to the fan ventilation of the building. The majority opinion, however, favored the fan rooms.

School 115, erected in 1914, was equipped with plenum fans, a humidifying pan and gravity exhaust ducts. Air flow was at all times ample. Little or no use was made of the humidifying pan. Respiratory sickness was greater in the fan ventilated rooms.

TABLE XXVII  
RESPIRATORY ILLNESS AT SCHOOL 115

VENTILATION TYPE	ABSENCE RATE	TEMPERATURE	RELATIVE HUMIDITY	PER CENT SESSIONS	
				ESP. FRESH	ODOROUS
B	30.4	67.6	28.	2	3
C	47.2	67.6	26.	1	6

(Note: The comparative data on sickness among pupils in School are not available for this school.)

A great deal of dissatisfaction with the fan ventilation existed among the teachers in this school. Complaints of dryness and drafts were common. The window rooms were by far the more comfortable.

In the three schools added in the second study, where Types B and C were studied, the window rooms showed less respiratory illness in two, and more in one. This supports the findings in the other schools.

In view of the favorable showing for fan ventilation in a very modern school with humidification such as 97, it may appear that the results of this study do not apply to installations of the latest design. It is true that we cannot generalize too widely in the matter, but on the other hand, School 59 possessed very modern equipment and was operated in every way as perfectly as 97, and yet the sickness rates here were higher than in the other rooms crudely equipped for window ventilation.

The buildings used were representative of what existed in New York City at the time. All were not of the very latest construction; nor were all of ancient pattern and design. All types were represented. The buildings were equipped and built with plenum fan ventilating systems. These systems were not ideal. They possessed faults as judged from the ventilating engineer's viewpoint of the year 1915.

The rooms fitted up for window ventilation were not built for this purpose. They were altered for the plan in mind. In no instance were the facilities complete.

As a comparison of average fan ventilation in New York City school buildings and ventilation of the same buildings without fans, the present study is entirely fair. If either type of room is lacking in its equipment, it is the window ventilated rooms which labored under the greater handicap.

#### METHODS OF RECORD TAKING

A word may be devoted here to the method of collecting records.

Each classroom was visited morning and afternoon. In the morning a record was made of the pupils absent and of the pupils in attendance who exhibited signs of a cold. The cause of absence was ascertained by a visit to the home. If a physician were in attendance his diagnosis was accepted. If no physician were in attendance, the nurse diagnosed the case, and when in doubt, her opinion was checked by a medical inspector. Many absences were not due to illness, as the subsequent records will indicate.

The diagnosis of minor illness among pupils in school was made by the

nurse. Many pupils with symptoms of a cold were pointed out to the nurse by the teachers.

At the beginning of the afternoon session the nurse again visited each room to ascertain absences, and the causes were determined in the usual manner. No effort was made at this time to determine illnesses among those present. A child recorded as having coryza in the morning was credited with the same affection in the afternoon if he or she were present in school.

In addition to the routine described above, the nurse entered each classroom at about 10:30 A.M. and 2:15 P.M., or just prior to the midsession aeration of the room, and recorded her impressions of the air conditions and made determinations of temperature and humidity with a sling psychrometer. The actual procedure was to enter the room, walk down the side aisle to the rear and thence up the middle aisle to the center of the room. The impression of odor was then recorded in terms of the scale given below. Next in order were recorded the sensations of temperature, moisture and air motion in terms as shown. The psychrometer was then swung at a level of about three to four feet from the floor, the wet bulb having been moistened from a small bottle of water carried for the purpose, and both wet and dry bulb readings noted.

ODOR	VOTING SCALES OF SENSE IMPRESSIONS						
		TEMPERATURE		MOISTURE		AIR MOTION	
Exceptionally fresh	1	Too warm	40	Moist	B	Dead	R
Odor absent	2	Satisfactory	30	Neutral	C	Bet. R & T.	S
Odor	3	Cool	20	Dry	D	Breezy or drafty	T

Before departing, the nurse made notations of the position of windows, whether open or closed, position of door and transom and activity of flag attached to the inlet register in the fan ventilated rooms.

Prior to the beginning of the study the nurses were carefully rehearsed in their duties and were given demonstrations in the use of the sling psychrometer.

The diagnosing of respiratory illness was frequently checked up by medical school inspectors and the supervising physician of the study, Dr. Leopold Marcus.

For several weeks during the second study all diagnoses were made by medical inspectors. Their opinions fully confirmed the interpretations made by the nurses and verified the casual inspections of physicians made prior to this time.

Respiratory illness is responsible for 19 per cent of absences from school; illness other than respiratory (including, however, the acute contagious diseases) 37 per cent; and causes other than illness 44 per cent.

#### THE SCHOOL PERSONNEL AND RESPIRATORY ILLNESS

The personnel of the pupils is a marked factor in determining the respiratory illness rates distinct from environmental influences. This was recognized at the outset, and an attempt, but partially successful, was made to equalize



this influence among the three ventilation types. Some schools were high in respiratory illness in both studies, while others were low in both.

Considering the total respiratory illness rates for both studies, Schools 12 and 147 had the lowest figures. These schools are on the lower East Side in the heart of the Russian-Jewish districts. The buildings are old. The average temperature was around 64 degrees. These two schools may be contrasted with Schools 165 and 115, which were located in very good neighborhoods and were attended by native born children of well-to-do parents. Both are of more recent construction than 12 and 147, P. S. 115 having been erected in 1914. In spite of environmental and social advantages, the upper Manhattan schools had high rates from respiratory illness.

In general, the schools located in congested districts and attended by pupils of inferior economic and sanitary status had less illness than those located in the better class neighborhoods.

Averaging the rates by social and economic status, the above facts stand out clearly.

SOCIAL AND ECONOMIC STATUS	SCHOOLS	RESPIRATORY ILLNESS RATES AMONG THOSE	
		ABSENT	IN ATTENDANCE
Very good	165, 115, 33	34.7	78
Good	59, 2 Bx., 51 Bx.	10.6	85
Poor	12, 147, 22, 97, 73	7.6	35.4
Very Poor	39	10.8	21.6

An explanation of this unexpected result is not easy to give. It prompts the query—Does prosperity undermine health and are our children of superior social status coddled to their detriment?

It is entirely possible that the children in the poorer districts acquire a more specific immunity by reason of their congested manner of living. It has been shown by Vaughan and the writer that the city boy made a hardier soldier in our army camps in 1917 and 1918 and was less subject to disease than the country boy.\*

It is also possible that the pupils in the poorer neighborhoods become "hardened" by slight exposure to cold, the body becoming less sensitive to environmental changes. Overheating and overeating are drawbacks to which this class is less familiar than their more fortunate schoolmates. In many respects material success converts a man into a less perfect physiologic machine. As the battle with the elements becomes less severe, the stimulation to physiological combativeness wanes. Lack of exercise, complex food as distinguished from simple coarse food, and life in uniformly and highly heated buildings without question weakens the body physically. In these statements may be found the explanation of this peculiar distribution of the ordinary forms of respiratory illness.

In view of the fact that the Type C rooms had more girls than the others, the question arises as to whether this would not account for the greater ill-

\*"Communicable Disease in the National Guard and National Army of the United States During the Six Months from September 29, 1917, to March 29, 1918." By Col. V. C. Vaughan and Capt. T. G. T. Palmer, *Jour. of Laboratory and Clinical Medicine*, Vol. III, No. II, August, 1918. Pages 693-698.



ness.\*\* This presupposes that girls are more susceptible to colds than boys, a supposition for which there is no justification so far as we know. There is no indication of this in the present study.

In the first study, P. S. 165, Type C had the highest respiratory absence rate, yet only 28% of the pupils were girls. The next highest rate was at P. S. 2, Bronx, Type B, where 50% of the pupils were girls. The third highest rate was at P. S. 165, Type B, where all the pupils were boys.

P. S. 2, Bronx, Type C, had high illness rates in the second study and yet only one-third of the pupils were girls.

P. S. 12 and 147 had low rates in both studies and the ratio of girls to boys was four to one in the first study and three to one in the second.

#### TYPES OF RESPIRATORY ILLNESS

Lacking the diagnosis of a medical man as to the specific type of respiratory illness in all instances of this study, we cannot publish these facts with any degree of certainty. However, we submit the statements of the nurses, which it will be remembered were frequently checked by the medical school inspectors.

The most prevalent form of respiratory illness causing absence was tonsillitis, which amounted to 32 per cent of the total. Slightly less prevalent were coryza (25%) and bronchitis (22%). Laryngitis and pharyngitis were less frequently mentioned.

Of the illnesses among those present in school, coryza representing 62% of the total, is the most prominent. Bronchitis stands second with 20% and laryngitis third with 10%.

Tonsillitis is especially prevalent in the Type C rooms. Other affections are greater in Type C, but no one affection seems to be characteristic of any ventilation type.

Illness other than those listed under the term "respiratory illness" were due to a great many causes. Diphtheria, scarlet fever, measles and chicken-pox were responsible for a small amount of absence, but absence from these causes combined amounts to less than that due to tonsillitis alone. In the first study the indefinite term "sickness" was given as responsible for more absence than any single cause. More specific terms were used in the second study. Headache was a frequent cause of absence, being mentioned most frequently in the Type C rooms. Appendicitis was responsible for a number of absences. A miscellaneous group of innumerable causes makes up the bulk of the absence causes. No particular form of illness seems to characterize any ventilation type.

#### WEATHER AND RESPIRATORY ILLNESS

Although aside from the ventilation question it is of interest to note the fluctuations in respiratory illness from week to week and the corresponding changes in weather. From the preceding pages it is evident that the indoor

\*\*The percentage of girls in Type C was 60 in the first study and 54 per cent in the second. The figures for Type B were 37 and 31 and for A, 41 and 49.

atmosphere has an influence on health. By combining the records of all schools it will be possible to follow the seasonal change in respiratory illness.

In Chart IV we have shown the sickness rate in each type of ventilation by weeks along with the room temperature and relative humidity. The data for Schools 97, 115 and 51 Bx. are omitted, as records were available only

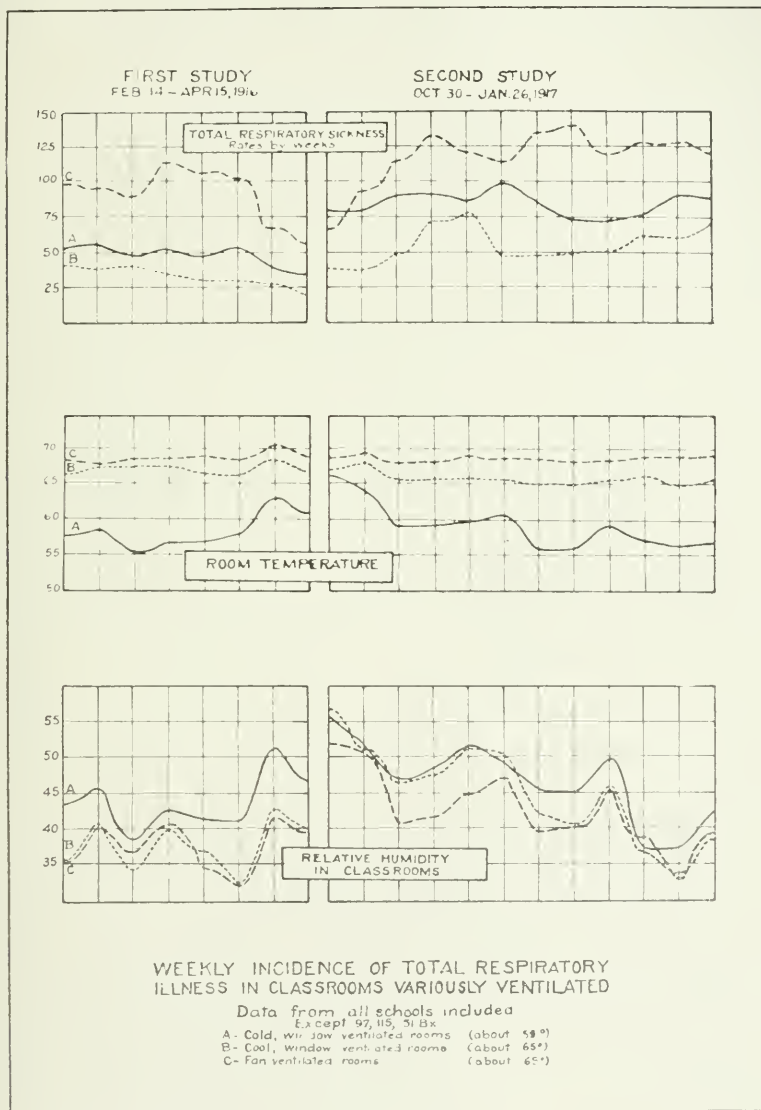


Chart IV.

during the last seven weeks of the second study. The fan ventilation group shows the greatest amount, and the window ventilated, moderate temperature, the lowest amount of respiratory illness throughout.

Respiratory illness declines from February to April although the fan ventilated rooms show the highest point during the middle of March. Colds

are at a higher level in October than they are in April. In the Type C and B rooms colds increase abruptly during the first 5 weeks in the Autumn. Type C then holds this level. The B rooms fall off and do not rise again until January. Colds in the Type A rooms increase gradually from October till

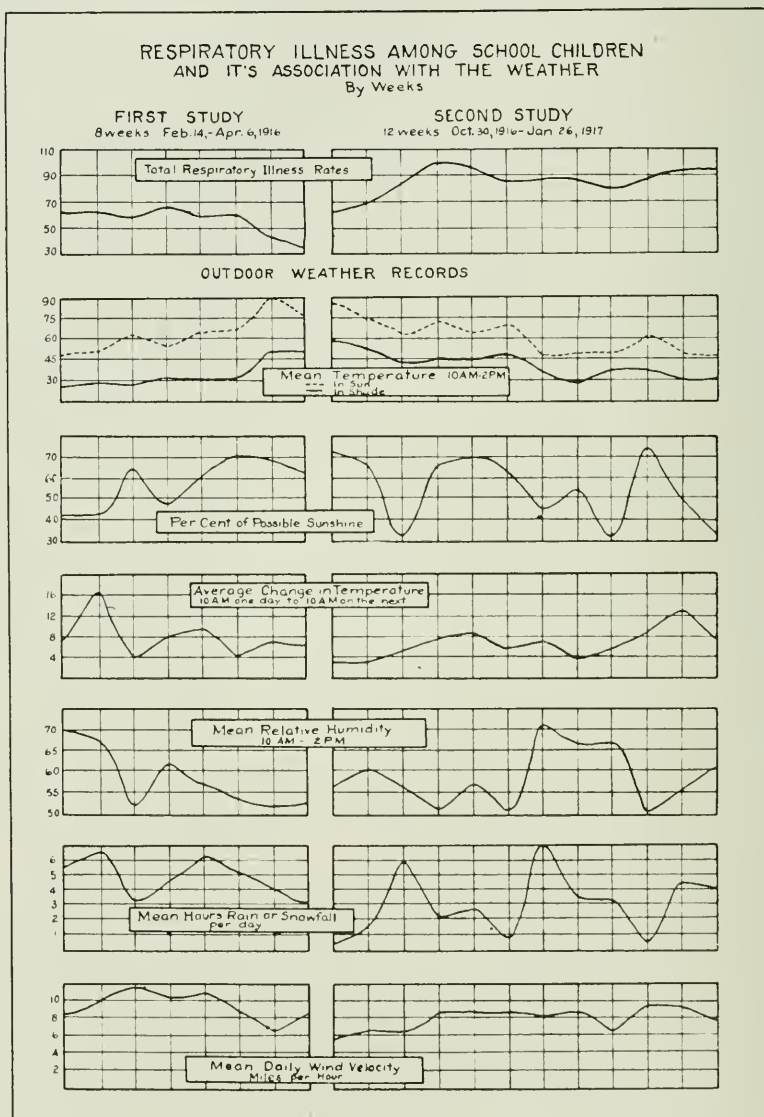


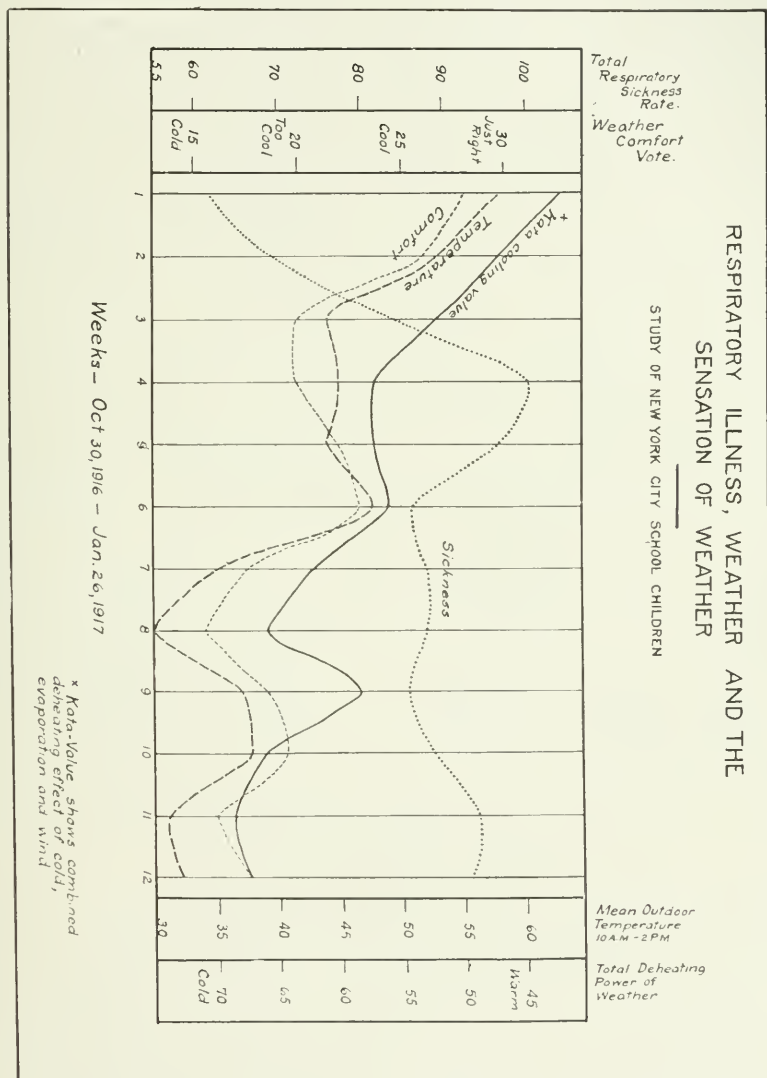
Chart V.

the first week in December. From this point they decline only to rise again the latter part of January.

Room temperature in the fan ventilated rooms is the most uniform, keeping quite constantly between 68 and 69 degrees. In the window rooms Type B, temperature has a slightly wider variation, from 65 to 68 degrees, but is consistently below Type C. In Type A rooms we note a wide variation,

from 55 to 66. During February and March these rooms run between 50 and 60, and this is true also in December and January. In April and November the temperature is from 60 to 65.

Relative humidity is greatest in the A rooms. It is lowest in March and January. As will be seen from a later chart, humidity is influenced mostly by outdoor temperature and next to that by outdoor moisture.



The general points to be noted from this chart are that colds are more common in fall than in late winter, that even though the room temperature and humidity in Types B and C are quite similar, there is an appreciable difference in the prevalence of colds, and finally that the course of respiratory illness from week to week is guided mainly by influences other than the temperature and humidity of the classroom.

That outdoor weather bears a close relationship to colds is illustrated by the various graphs on Chart V. Far overshadowing other weather influences is temperature. In the spring, colds decrease as the weather becomes warmer. In the fall, oncoming cold weather is coincident with increasing respiratory affections.

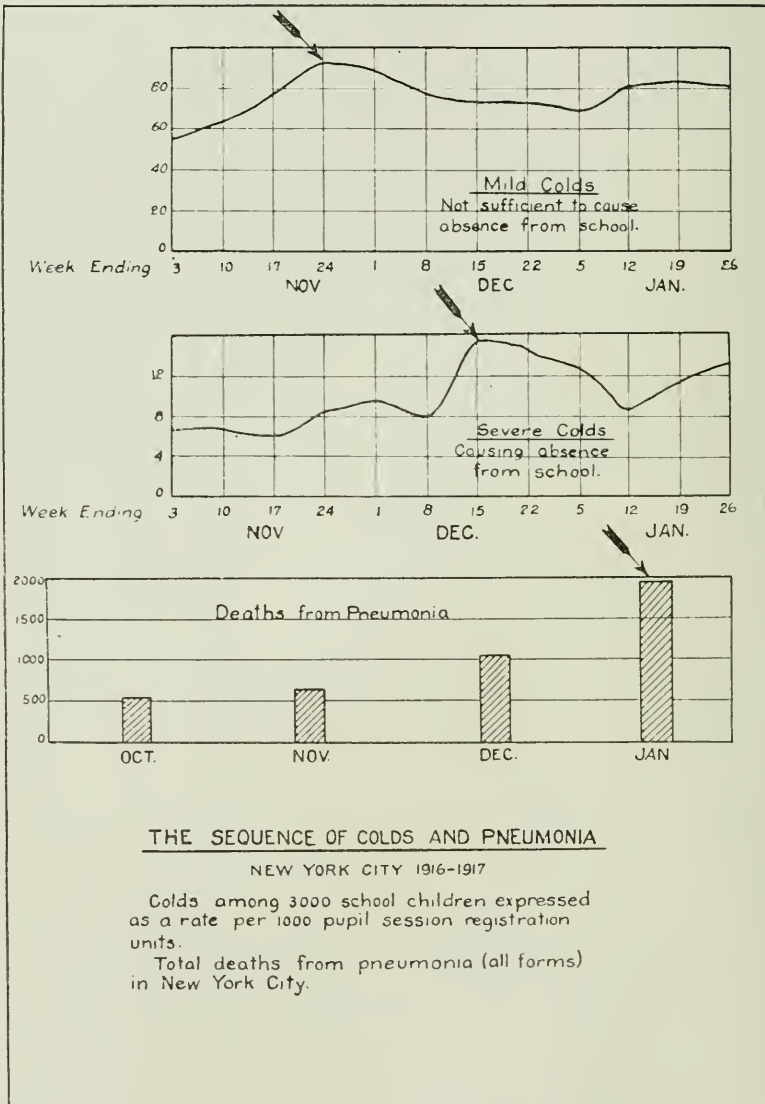


Chart VII.

What is understood as changeable weather does not have marked influence on colds. The third week in February shows an extreme daily change in temperature with no accompanying increase in colds.

Relative humidity shows wide variations from week to week without corre-



sponding undulations in the sickness curve. The late winter is more blustery than the fall, and yet colds are less numerous in the windy season.

Temperature and colds are most closely related. Is it temperature *per se* or the total chilling effect of all weather elements combined? The formula derived by Dr. Leonard Hill of England from his instrument known as the kata-thermometer permits us to sum up in a single expression the combined deheating effect of cold, evaporation and wind action.\* From the mean daily outdoor temperature, vapor pressure and wind velocity we have computed the mean "total H" or total deheating power of the elements. This graph has been placed alongside curves for respiratory illness in the second study, and mean temperature and a fourth curve labelled "comfort." The comfort vote is a summary of the recorded daily impressions of the temperature feeling of the weather, as judged by three members of the staff of the Ventilation Commission.

The kata and temperature curves closely parallel each other. In some respects the kata curve is more closely associated with the rise and fall of illness than the temperature curve. Thus, the kata curve shows increasing cold for the first four weeks corresponding to increasing illness. The temperature curve shows no increase in cold in the fourth week over the third.

Illness falls off as the temperature stabilizes. In the 7th week temperature again descends, and sickness picks up. The 10th week is much colder as judged by the kata reading although the temperature is no lower. There is however, very low humidity and high wind velocity, which are heat extractors. These data suggest that it is the total chilling effect of the atmosphere rather than low temperature alone that is conducive to illness.

The curve of outdoor temperature comfort closely parallels the temperature curve, more so than the kata curve.

The sequence of mild colds in November, followed by heavier colds in December and then by pneumonia in midwinter, is strongly suggestive of a progressive weakening of vitality. Our mid-winter pneumonia peak is thus the result of attrition of vital resistance caused, among other things, by acute respiratory affections in the months preceding.

#### SUMMARY AND CONCLUSIONS

From the results of this study there appears to be something inherent in the indirect method of ventilating schoolrooms by means of forced draught and gravity exhaust, as practiced in this study, that is productive of respiratory affections, something which is not present in rooms ventilated with windows and gravity exhaust. What these unfavorable elements are is not entirely clear. Higher temperature is one. Uniformity of temperature and air flow is another. Uniformity is characteristic of the fan ventilated room. In an unvarying atmosphere the occupants miss that pleasant stimulating effect. Evidently the absence of this quality affects health adversely as well as comfort.

\*"The Measurement of the Rate of Heat loss at Body Temperature by Convection, Radiation and Evaporation." By Leonard Hill, F. R. S., O. W. Griffith and Martin Flack, *Philosophical Transactions of the Royal Society of London*, Series B, Vol. 207, pgs. 183-220, 1916.

The temperature of window ventilated schoolrooms may be reduced as low as 59 degrees without increasing the prevalence of colds.

It must not be inferred that window ventilation as represented in this study was uniformly satisfactory. It was not. As a rule the rooms exposed on the east do not fare as well as others. Ample exhaust openings are better than those of small area. There is the matter of location of outlets with respect to the windows, location, size and control of direct radiation, window deflectors, etc., which affect the success of window ventilation. All of these factors must be studied.

In spite of our inadequate knowledge of window ventilation at its best, the fact remains that the window rooms of this study, even though of crude arrangement and not built originally for the purpose, competed on favorable terms, from a hygienic and aesthetic standpoint, with the most elaborate and costly fan and duct equipment. The tendency in the past twenty years has been away from natural and toward mechanical ventilation. The time and effort of the heating and ventilating engineer has been directed toward the perfecting of mechanical means for aerating buildings. What would the same amount of effort have yielded if expended on the development of natural ventilation? Possibly something of great value and at less expense.

Because window ventilation is practicable for the ordinary schoolroom, it does not follow that the assembly room, the theatre and other places seating several hundred people can also be dealt with in this manner. Each type of enclosure must be handled as a distinct problem. Natural ventilation has its limitations. That the schoolroom is not beyond these limitations is the indication of this study.

The factors which, above all others, promote comfort, health and efficiency are coolness and fluctuating air motion. If the teacher maintains her classroom in a changing condition, without draughts, between 64 and 70 degrees, with the mean lying nearer the lower figure, it matters little from a practical standpoint what the other measurements of ventilation indicate. To this standard the room properly equipped with window ventilation and gravity exhaust, can readily conform.

In its quantitative effect on respiratory illness school ventilation is of much less moment than the outdoor weather influence. Respiratory affections increase with the onset of cold weather. They diminish with the advent of mild weather in the spring. Wind and humidity accentuate the temperature influence. Sunlight exerts at least a warming influence sufficient to modify the unfavorable effect of cold. Abrupt changes in temperature do not influence respiratory illness as much as one might expect from everyday experience.

The sanitarian is interested in the prevention of the tremendous increase in the mortality from the pneumonias, which occurs in cold weather. If it is possible to mitigate the unfavorable weather effect by the proper regulation of the indoor atmosphere, it behooves the public health fraternity to bend every effort toward this goal. The problem is an alluring one. The results of this school study hold forth much promise in this direction.

## LABORATORY METHODS

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### AN APPARATUS FOR THE RAPID DETERMINATION OF NITROGEN IN BLOOD AND URINE\*

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BY C. E. REYNER, DETROIT, MICH.

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THE adoption of Folin and Wu's excellent system of blood analysis<sup>1</sup> in this laboratory created the demand for a device by which a large number of total nonprotein nitrogen determinations could be made simultaneously. With the idea that the device would be used also for the determination of total nitrogen in urine, an apparatus has been devised and made which has given a high degree of satisfaction. Twelve specimens of blood or urine may be digested simultaneously and the determinations be completed with ease in thirty

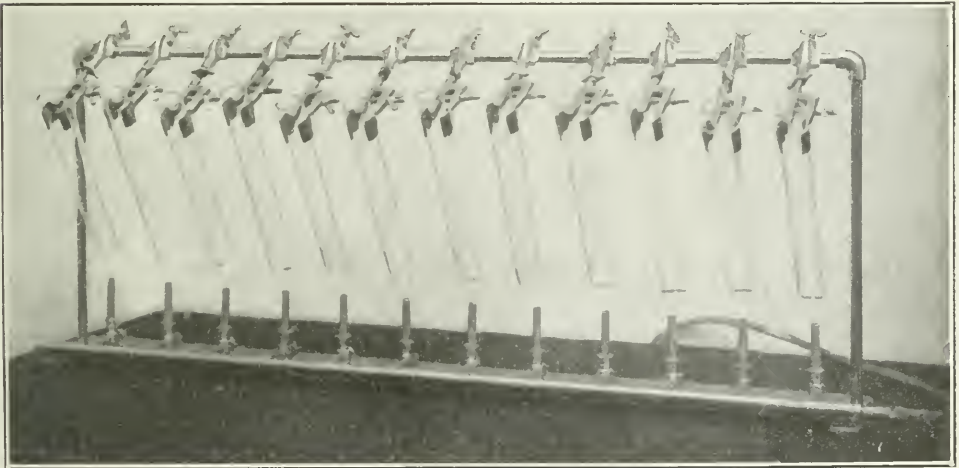


Fig. 1.

minutes. Devices for this purpose heretofore described have been inefficient in that a long period of time was necessary to complete the series due to long periods of digestion and to the numerous transferences of solutions. The much used macro Kjeldahl battery of digestion flasks and distilling flasks requires at least two hours to complete a series of twelve determinations in addition to requiring large amounts of acid and caustic soda. Thus, the apparatus described later effects a great saving in time as well as in chemicals. So far as accuracy is concerned, by using Folin's technique for urine<sup>2</sup> highly satisfactory recoveries of added nitrogen have been obtained by the author, as accurate as the macro Kjeldahl method if not more so.

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\*From the Laboratory Department, Henry Ford Hospital, Detroit, Mich.

The apparatus which is illustrated in the accompanying photograph consists of the base plate, 31.5"x3"x0.25", into which 12 micro burners are serewed, 3 pieces of  $\frac{3}{8}$ " pipe, 2 elbows, 12 clamps, 12 Pyrex tubes, 8"x1", and one feed pipe, 33"x0.5", to supply gas to the burners. At each end of the base plate is a small cross piece, 5"x1"x0.25", to increase the stability of the device. The type of micro burner necessary is that which has a removable base. After removing the bases, the burners are serewed into appropriate holes in the base plate and connected suitably to the feed pipe. The frame supporting the clamps and tubes is 12" high. The clamps are adjusted so that the end of the tube is about one inch above the burner. Any change in the angular adjustment of the clamps is unnecessary after the first adjustment has been made, i.e., no adjustment need be made during the digestion. The plate, frame, feed pipe and clamps are steel, but, to give a more permanent and durable finish, these parts have been nickel plated. The micro burners need not be plated as they are usually made of a very durable metal or alloy. It is very convenient to have at hand with this apparatus a test tube rack capable of holding 12 tubes, 8"x 1".

Two inch glass funnels have been found most convenient to use in covering the mouths of the digestion tubes during the final heating.

Langstroth<sup>3</sup> found difficulty with the digestion of the blood filtrate and suggests that the tube be held as nearly horizontal as possible during the preliminary heating and then changing to a vertical position for the final heat treatment. With the apparatus described no such difficulty has been encountered and no change in position of the digestion tube has been found necessary. It is only necessary to reduce the flame for the final heating. Probably his use of m-phosphoric acid as the precipitant caused his difficulty with the digestion.

#### REFERENCES

<sup>1</sup>Folin and Wu: Jour. Biol. Chem., 1919, xxxviii, 106.

<sup>2</sup>Folin: Jour. Biol. Chem., 1916, xxvi, 473.

<sup>3</sup>Langstroth: Jour. Biol. Chem., 1918, xxxvi, 377.

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## AN AUTOMATIC PIPETTING DEVICE\*

By W. F. LORENZ, M.D., MADISON, WIS.

IN THE serological laboratory of the Wisconsin Psychiatric Institute a large number of Wassermann tests are made every day. In our method eight tubes are used for each serum. With an average of about one hundred serums daily it means the addition of measured amounts of fluid to over eight hundred test tubes. Certain of the factors that enter into the Wassermann are always used in constant amounts. For instance, the dose of corpuscle suspension is fixed according to whatever system is employed. In our method the amboceptor dose is likewise constant since we adjust the hemolytic system by

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\*This apparatus was developed during a research on treatment of central nervous syphilis undertaken at the University of Wisconsin by funds appropriated by the Interdepartmental Social Hygiene Board of Washington, D. C.



varying the amount of complement. The dose of antigen is also fixed in the system we use. Our technic therefore requires the delivery of constant measured amounts of three elements into a series of test tubes. This work is ordinarily done with a graduated pipette. The corpuscle suspension in a 1 per cent strength is measured by means of a 10 c.c. pipette. The amboceptor and antigen used in smaller doses are measured by a 1 or 2 c.c. graduated pipette. This is a time-consuming and laborious task.

In an effort to improve this technic an apparatus used in the department of Pharmacology at the University of Wisconsin was tried. This apparatus was built by J. S. Hipple and patterned after the Woodyatt<sup>1</sup> machine used for the constant intravenous administration of glucose. After a satisfactory trial a further modification was designed and built by Mr. Hipple, to meet the special

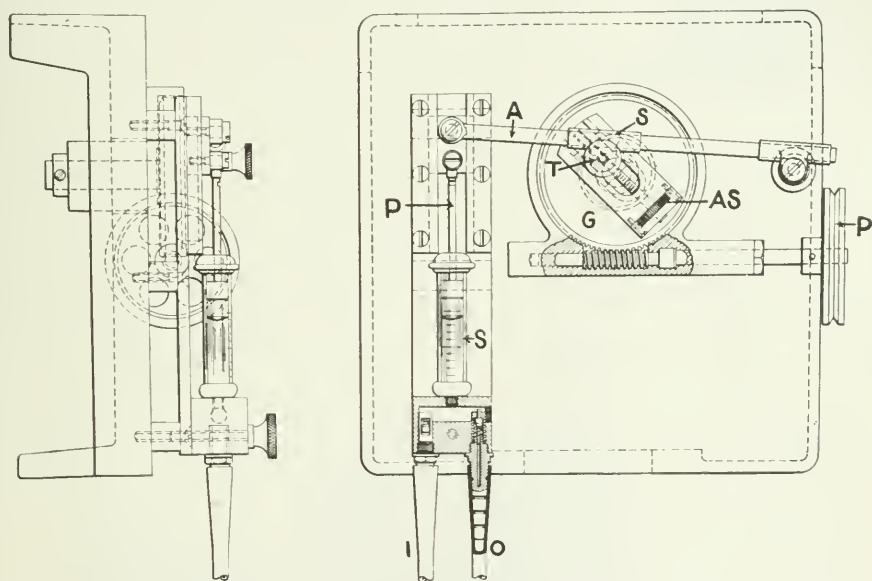


Fig. 1.—S, 2 c.c. record syringe; I, inflow; O, outflow; P, syringe piston connections with slide; A, connecting rod; S, movable sleeve on connecting rod; T, thumb screw to fix eccentric position; AS, adjusting screw controlling eccentric position; P, pulley for power transmission or direct motor attachment.

needs of our laboratory. This latter instrument has now been in constant use for over six months and has been very satisfactory.

The apparatus consists of a mechanically operated syringe in which the inflow and out-flow of fluid is directed by two simple check valves. The syringe piston is attached to a connecting rod pivoted at one end. At about the middle of this rod is a sleeve which is attached to a gear. This attachment is movable, affording any desired eccentric position; the farther from the center the greater the movement of the connecting rod and accordingly a larger stroke of the piston. Precise adjustment can be made and the instrument is calibrated at this part. The adjustment is made with the adjusting screw and firmly held in position by a thumb screw. The syringe used in our work is a 2 c.c. record. The large gear is operated by a worm, the shaft of which is attached to either a small motor as direct power or a pulley for power transmission from a counter shaft. The



speed of the apparatus is controlled by a rheostat if directly connected to a motor.

Two such instruments have been in constant use for several months. At various times a careful test was made. This consisted in taking a series of alternate deliveries into weighing bottles. Each delivery was then weighed on an analytical balance. The machine was set for either 1 c.c. or 0.5 c.c. One c.c. of saline was carefully measured with an Oswald pipette and taken as a standard. The temperature of the saline was constant under the conditions of the test.



Fig. 2.

Another series of tests were made with a 1 per cent corpuscle suspension. The variation in the deliveries of the apparatus never exceeded 0.015 grams per c.c. That is the instrument would occasionally deliver 0.0075 gms. more than the standard and during the same trial without further adjustment deliver an amount which was .007 less than the standard. The maximum variation from the standard was therefore less than 0.02 c.c. This degree of accuracy was obtained under a speed of 48 deliveries per minute. At a slower speed the varia-

tions from standard were still less. Trials were not made at a slower speed than 20 per minute.

In testing the accuracy of hand pipetting with a 10 c.c. graduated pipette under conditions of greater care and precision than can be routinely employed, the variation in amounts delivered was twice as great and in some instances four times. In other words a variation of 0.05 c.c. is not unusual in the routine use of a fairly large calibered 10 c.c. graduated pipette. The apparatus therefore is without question as accurate as the best hand technic possible in the usual serologic technic. This advantage would in itself not be sufficient. The gain lies in the speed with which these accurate deliveries are made. In a series of 80 tubes to be filled with 1 c.c. amounts the hand method, taking into account refilling the pipette, consumes four minutes. The apparatus fed from a constant supply in an overhead container, will perform the same task in one minute and forty-five seconds. This gain in speed becomes a big factor when several racks are run at one time and the operation is repeated throughout the series of tubes. The total gain in time becomes a matter of hours when a large series of tests are made.

This apparatus is therefore recommended for any technical procedure where constant amounts of fluid are to be measured and delivered at intervals and at a controllable speed.

#### REFERENCES

- <sup>1</sup>Woodyatt, R. T., Sansum, W. D., Wilder, R. M.: Collective Reprints, also S. A. Sprague, Memorial Institute, Vol. iii, 1915.

## A MODIFICATION OF THE KJELDAHL STILL FOR DISTILLING LARGE QUANTITIES OF WATER\*

BY C. E. SWANBECK, M.D., CLEVELAND, OHIO

THE still which is described in the following paragraphs makes use of the Kjeldahl distilling apparatus for furnishing the heat and for condensing the steam generated. The Kjeldahl is seldom used routinely

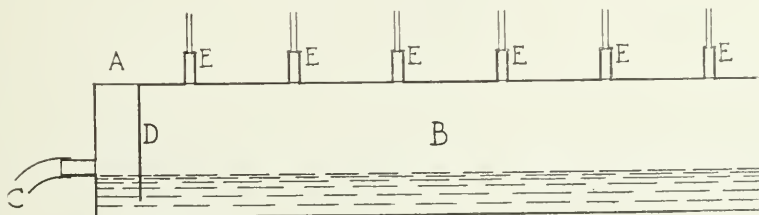


Fig. 1.

since colorimetric methods are more simple to perform. For this reason our Kjeldahl outfit is idle for considerable periods of time. In order to make more

\*From the Laboratories of Mt. Sinai Hospital, Cleveland, Ohio.

use of it, I have devised a copper box which can be quickly connected and disconnected to the Kjeldahl so that it now can be used for distilling large quantities of water. The copper box for generating the steam is shown in Fig. 1.

A is an opening at one end on the top into which the overflow of water from the Kjeldahl condenser empties. This water is heated by the condensing steam in the coils and therefore any gases in solution are liberated when they come to

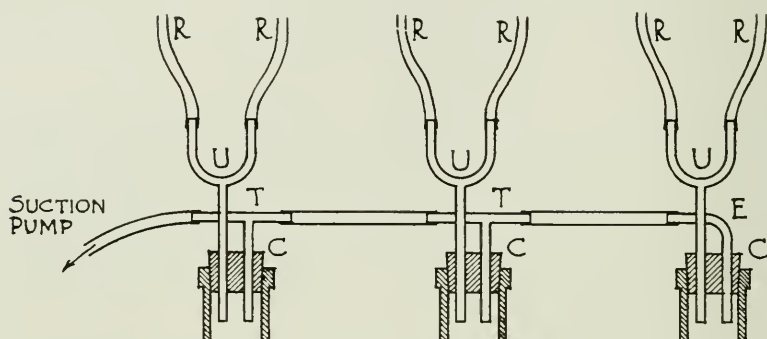


Fig. 2.

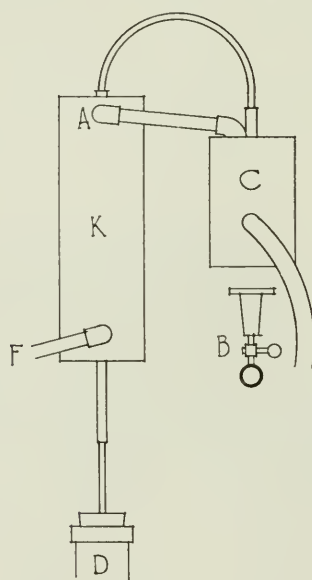


Fig. 3.

the opening A. The chamber B fills with this preheated water until it reaches the level of the overflow pipe C. The water now stays at this level and the water coming in at A does not reach the chamber B except as it gets below the level determined by the opening at C. The copper apron D extends below this water level so that the steam generated in B cannot escape at this end, instead it passes out through the openings marked E on top of the box. There are six openings corresponding to the six coils of the Kjeldahl. Into each opening is placed a cork and the connections from the coil are fitted into these corks. This

connection leads the steam from the chamber *B* into the coils of the Kjeldahl where they are condensed. And as the water is formed, it is led into three large bottles through connections as shown in Fig. 2.

The rubber stoppers *C* have two holes bored in them, one for the glass tubing shaped like the letter "U" and marked "U" for leading the distilled water into the bottles. The other hole is for the glass tubing shaped like the letter "T" and marked "T" which are connected with each other as shown in Fig. 2 and the last one is shaped like an elbow. This series of connections is attached to a suction water pump in order to create a partial vacuum in the three bottles, in which the distilled water is collected.

Fig. 3 shows the side view with all connections made. *K* is the Kjeldahl still: *A* shows the pipe which leads the overflow water from the Kjeldahl into

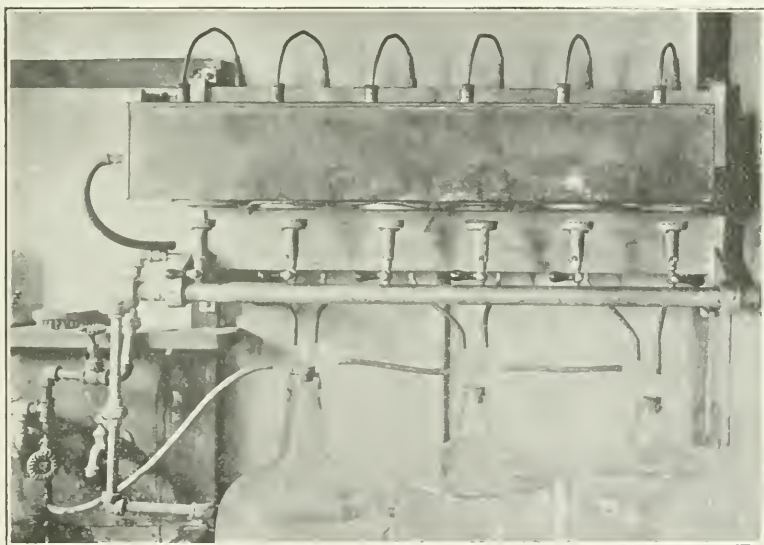


Fig. 4.

the copper boiler, *C*; *F* is the pipe connection to the tap water faucet: *B* is the bunsen burner for supplying the heat.

We attached the suction pump to the bottles as shown in Fig. 2 in order to lower the atmospheric pressure within the bottles and this also lowers the atmospheric pressure in the copper boiler. Water boils at a lower temperature when atmospheric pressure is reduced and conversely the steam generated at this temperature takes less cooling to be condensed and form water. This simple attachment adds greatly to the rate of distillation. The more the atmospheric pressure is reduced, the faster the distillation and the less heat required to bring the water to boiling.

Fig. 4 shows the entire still assembled as it is used in our laboratory. We have placed a strip of sheet iron under the copper boiler and above the bunsen burners to prolong the life of the still.

# *The Journal of Laboratory and Clinical Medicine*

VOL. VII.

OCTOBER, 1921

No. 1

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.

Washington, D. C.

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## EDITORIALS

### *Vitamins and Deficiency Diseases*

CONTROL of diet is one of the most important therapeutic measures in the hands of the physician. It is very often of greater importance than the administration of drugs and yet how seldom is it the case that it is given anything like as great a prominence in treatment as the latter. One reason for this is no doubt the unsatisfactory and indefinite state in which our knowledge of dietetics has been until recently. Foods usually develop their effects on the body slowly and imperceptibly; drugs usually do so quickly and with immediate results. Cause and effect are evident in the one case but obscure in the other, and it has consequently taken much longer for our scientific knowledge of dietetics to gain that degree of precision that it is necessary before it can be put into practice in the treatment of disease.

During the past decade more has been added to our knowledge of the relationship of foods to the well-being of the animal body than in all preceding times. The prominence given to the importance of the calorie value of foods by the work of Rubner in Germany and, later, of Atwater, Benedict and Lusk in this country, did not minimize the importance of an adequate proportion of protein, the reason for which was first clearly shown by the



work of Osborne and Mendel, to be that an adequate amount of the various amino acids should be assimilated for synthesis of the body protein. But it soon came to be recognized that although several conditions of semistarvation, simulating those met with in clinical practice, can be attributed to inadequacy in calories or in amino acids yet there are others, scurvy for example, which in some way are also related to the diet, though not to its calorie value or protein content. This more subtle, less tangible property is now known to be the vitamin content of the food. The first clear recognition of vitamins dates to the discovery by Eijkman that the tropical disease known as beriberi is almost peculiar to localities in which the diet is very largely composed of highly polished rice and that the disease is cured when the huskings, which are removed by polishing, or an extract of them is added to the polished rice. Since this discovery many investigators (Gowland Hopkins, Funk, McCollum, etc.) have devoted their attention to the subject of vitamins and enough precise knowledge has been accumulated to enable the physician to apply some of it with great effect in his daily practice. That this may be done profitably, however, requires a greater attention to details than it is the custom of physicians to give to matters of diet, and it is probable that it will be some time yet before the relationship between vitamins and disease comes to be adequately recognized.

A most valuable contribution, bearing on these relationships has just appeared as a monograph entitled "Studies in Deficiency Disease" by Col. McCarrison of the Indian Medical Service.<sup>1</sup> Before we proceed to indicate some of the more important facts presented in this volume it may be well to point out that throughout the whole of it insistence is put on the necessity of remembering that although it may often appear as if some obvious dietetic deficiency, for example, of one of the vitamins, is the specific cause of some particular disease, the other properties of the diet, such as the calorie value, the proportion of the proximate principles and the amount of inorganic salts, must also be taken into account.

The greater number of the observations recorded in this book relates to the effects of the three vitamins namely:

- (1) Vitamin B (or water-soluble food factor, or antiberiberi vitamin)
- (2) Vitamin A (or fat-soluble food factor, or antirachitic vitamin)
- (3) Vitamin C (or antiscorbutic vitamin).

Diets containing varying proportions of these vitamins, as well of the proximate principles of food (proteins, fats and carbohydrates) were prepared by using carefully selected mixtures of foodstuffs. Thus, to obtain a diet consisting practically solely of carbohydrate with no vitamin, autoclaved rice was used; to obtain one containing carbohydrates, fats and vitamin A, rice *plus* butter, or one also containing vitamin C rice *plus* butter and onions, or one containing everything necessary save vitamin B, autoclaved food *plus* onions and butter.\* The mixtures of foods were fed to groups of pigeons, monkeys and guinea

\*The proper choice of these food mixtures was made possible by consulting the results of the numerous investigations which have been made to determine exactly how the vitamins are distributed among the various foodstuffs. Simple tables showing these results are now readily available and should be in the possession of every physician.<sup>2</sup>

pigs, each group being composed of several individuals and the feeding of each group being maintained of the same character throughout the observation. The body weight of the animals was taken at regular intervals, the general condition closely watched, and the time of appearance of definite symptoms carefully noted. So far the work is very much like that of other investigators in this field, but the most noteworthy feature of McCarrison's researches is that the pathologic condition of the various organs and tissues was very carefully studied, either in animals that had died as a result of the food deficiency, or in animals killed at a stage when the earliest symptoms made their appearance. In this regard, the present investigation is much more complete than any other with which we are familiar. It is the work of the clinical pathologist as compared with that of the biochemist and is accordingly all the more important from the physician's standpoint. The biochemist has indeed blazed the trail into the unexplored regions of dietetics and now the clinical investigator must follow to build a solid road along which every physician may travel.

By using such diverse groups of animals as pigeons, guinea pigs and monkeys for the experiments, liability of error in the application of the results to explain deficiency diseases in man is greatly minimized, for, if a certain condition is found in all three groups of animals constantly to result from some definite dietetic fault, it is unlikely that man will be an exception.

In the first part of the monograph the time of onset of symptoms, the rate of decline of body weight and the time of death resulting from food deficiencies are more particularly considered and it is clearly brought out, especially in the observations on monkeys, that "two factors (are concerned) in the causation of morbid states resulting from food deficiencies: (1) want of vitamins, and (2) want of balance of the food with respect to proximate principles—carbohydrates, fats, protein and salts."

In the second part, the other factors influencing the onset of deficiency disease, and its symptomatology, are discussed. Such factors as race, age, sex, previous food conditions, fatigue, damp, mental state, overcrowding, etc., are all shown to have an influence, and in the summary to this part it is pointed out that it is especially important "to realize that persons receiving too little vitamins are living in a state of potential morbidity which may be converted into one of actual disease by a variety of factors which further exhaust metabolism." Variability of different individuals to the same degrees of food deficiency is also insisted on.

Not the least important point made in connection with symptomatology is that more attention should be given in experimental work on animals to the premonitory signs, for it is often these, rather than the terminal symptoms, that simulate clinical conditions in man. Thus, when monkeys or pigeons were fed with food lacking vitamin B, the first symptoms to appear were those referable to the digestive and endocrine systems and only later did those referable to the nervous system—polyneuritis—manifest themselves. In monkeys these symptoms appeared in a definite sequence and they included

loss of appetite and distaste for food, indigestion and colitis, loss of weight and of vigor, anemia, unhealthy skin, a tendency to edema and finally subnormal temperature and cardio-vascular depression. It was only after these symptoms had been present for some time that symptoms attributable to malnutrition of the nervous system made their appearance. Similarly when vitamin A was deficient, failure of the processes of growth, susceptibility to infections and eye symptoms were usually present, and it is suggested that such clinical conditions as rickets, osteomalacia, deficient calcification of the teeth and possibly phosphatic urinary calculi may be due to a lack of this vitamin in the diet of man.

The most original part of the research (part III) is that relating to the pathogenesis of the conditions brought about by the faulty diets. In order to furnish standards for comparison, the effects of complete starvation are first of all considered, the weight of the various organs being recorded in tables and charts and their macroscopic and microscopic appearances carefully described. In pigeons, for example, it was found that all organs except the adrenal glands and the brain decrease in weight, when compared with those of healthy birds. The adrenal glands actually increased in weight. When a diet composed exclusively of milled rice was given (i.e., one free of all vitamins and containing a great excess of carbohydrate), the same changes were seen but in a different degree, due to the fact that inanition in this case is more prolonged. The atrophy of the thymus and testicles was especially prominent. By adding butter and onions to the rice diet (i.e., vitamins A and C, also fat), "the general appearances were similar to those of birds fed exclusively on autoclaved rice" but "the heart, the thyroids, the spleen, the testicles and the pituitary body are smaller and the liver, the adrenals, the ovary and the brain are larger." These results show that the changes due to *avitaminosis* are similar to those of inanition and that it is absence of vitamin B that plays the prominent part in their production. But just as this starvation occurs when vitamin B is absent, although the diet may contain superabundance of calories, so also will it occur when there is abundance of vitamin B but a shortage of calories. Calories and vitamin B are equally important in maintaining the nutritional balance. In similar observations on monkeys the diets fed to different groups were autoclaved rice, this *plus* butter, autoclaved food *plus* onion and this *plus* butter. It was found that the changes in the weight of organs corresponded to those of deficiently fed pigeons although they might differ in degree. The adrenals and pituitary became hypertrophied and the other organs atrophied but the testicles relatively less so than in pigeons. The brain was found to be increased in weight on diets excessively rich in butter. Taking the results as a whole the most outstanding conclusion is that the adrenals become enlarged by malnutrition "whether this is due to deprivation of calories or to the state of inanition resulting from foods deficient in vitamins and ill balanced in other respects." Considerable variations were found in the degree of change in weight of the organs of different individuals.

Coming now to the pathologic changes found in the various organs, the most important were found in the gastrointestinal tract, the endocrine organs, and the central nervous system. In pigeons fed on autoclaved rice there was atrophy of the coats of the bowel and in 70 per cent of the 152 birds examined, very striking pathologic changes as well. These included congestion and hemorrhage, atrophy of the myenteron, degenerative changes of the plexus of Auerbach, atrophic and inflammatory changes of the mucous membrane and of the lymphoid structures. Evidence of changes favoring systemic infection were also obtained, such as bacterial invasion with "intense inflammatory and necrotic changes in the mucous membrane and underlying coats of the bowel." The clinical significance of this observation need scarcely be emphasized. It is further noted "that the mucous membrane may be so destroyed and the changes in the bowel so pronounced as to render recovery impossible." This no doubt explains why some pigeons showing symptoms of polyneuritis recover when given vitamin extracts while others show no improvement. When butter and onions were added to the rice diet the above changes were less frequently encountered but the addition of butter alone did not minimize the severity of the changes. In guinea pigs fed on a diet of crushed oats and autoclaved milk (deficient in vitamin C and in salts), hemorrhage infiltration and degenerative changes in the myenteron and myenteric plexus were as marked as in pigeons fed on autoclaved rice. In concluding the description of this observation, McCarrison remarks that "the functional perfection of the gastrointestinal tract is dependent in considerable measure on the adequate provision of vitamins and salts found in fresh vegetable foods; a fact which provides an explanation of the beneficial action of orange juice on the digestive and assimilative processes in bottle-fed infants." He points out also that the intestinal lesions were present in the majority of cases before clinical evidence of scurvy was present (it being of course vitamin C that is mainly concerned), and he suggests "that the symptoms of scurvy as described in textbooks are the grosser evidences of a disordered state of metabolism, the minor or pre-scurvitic manifestations of which are probably frequently overlooked, especially in children." In monkeys the gastrointestinal changes are particularly important. Feeding on autoclaved rice caused the colonic mesenteric glands to become greatly enlarged, indicating an intense degree of toxic absorption, and the small intestine to become greatly thinned, often more so in certain portions than in others, with ballooning (usually in the ileum) and, frequently, with intussusception. The large bowel showed a thinning of the walls and remarkable atrophy of the longitudinal muscle band with consequent loss of the normal puckerings; ballooning was also frequent. Congestive and necrotic changes were also marked, especially in the duodenum and large intestine; indeed, in the latter typical colitis without ulceration was the rule. The histologic changes though in general the same were somewhat different in detail from those seen on similarly fed pigeons, especially with regard to the locus of the hemorrhages. The changes were such as would greatly favor hemie infection from the diseased bowel. When in place of rice autoclaved food was fed, (i.e.,



deficient only in vitamins) the gastrointestinal lesions were still extreme, colitis and ballooning being very frequent although intussusception was less so. "The colitis was invariably associated with enlargement and discoloration of the mesenteric glands." It is important to note that the addition of onion (vitamin C) did not improve the diet. The addition of butter and onions (vitamins A and C) however considerably reduced the frequency of gastrointestinal lesions. The conclusion drawn is that "the health of the gastrointestinal tract is dependent on an adequate provision of accessory food factors, especially vitamins B and C." Absence of vitamin B is apt to produce colitis and absence of vitamin C is especially associated with congestive and hemorrhagic lesions in the tract. These effects of vitamin deficiency become accentuated when there is excess of starch or fat, or of both in the food or when this is ill-balanced in other respects.

In introducing an account of the effects produced on the endocrine organs, the similarity of action between vitamins and hormones is pointed out. The thyroid glands were found to atrophy in pigeons and monkeys both when the animals were completely starved and also, although to a lesser degree, when fed on any of the imperfect diets. Histologically, the glands were but little affected, moderate congestion and necrobiosis being sometimes observed. Complicating infections were common, however, indicating that in consequence of the food fault these glands are rendered susceptible to injury by bacterial and toxic agencies. The parathyroids sometimes showed the presence of hemorrhages especially when the food was excessively rich in fats and starch. The pituitary gland was hypertrophied in pigeons and monkeys especially males, in consequence of food deficiency but the histologic changes (of the pars glandularis) were not striking.

The most important changes were observed in the case of the adrenals. In all those classes of animals enlargement was found both in complete inanition and in any of the forms of partial food deficiency that were studied. This enlargement showed a tendency to be more marked when the food was disproportionately rich in energy-bearing constituents. Histological examinations showed in pigeons that the cortical columns formed a greater proportion of the section than normal and the lipoid material in their cells was apparently diminished. There was little change in the amount of chromophil material in the medullary cells although there was more or less engorgement in the region of these cells. In guinea pigs, the histological changes resulting from a scorbutic diet (crushed oats and autoclaved milk) were particularly hemorrhagic, infiltration and degenerative changes in the cells of both medulla and cortex. The hemorrhages were confined to the cortex and occurred even when there were no indications of scurvy in other parts of the animal's body. Of still greater importance, as indicating the nature of the changes occurring in the adrenals, are the results of measurement of the amount of epinephrine (adrenalin) extractable from the altered glands. These measurements were made either by preparing saline extracts and observing the effect which they produced on the arterial blood pressure on intravenous injection (in sleep),



or by assaying the amount in weak acid extracts by the colorimetric method of Folin, Cannon and Denis, or by using both methods. It was found that the enlargement of the adrenal was associated with a full load of epinephrine (i.e., the amount per gram of enlarged gland was the same as for a normal gland), when the food contained no vitamins but that reduction of this hormone occurred (per gram of enlarged adrenal) when a diet of autoclaved rice and butter was fed, and also on a diet deficient only in antiscorbutic vitamins. In the last mentioned case (in guinea pigs fed on oats and autoclaved milk) the total quantity of epinephrine in both glands was less than one half that present in healthy pigs although the weight of the organs was more than double.

The observations on the changes in the adrenal glands are given side by side with those on malnutritional edema because, as the author says, "of the intimate association which has been found to exist in pigeons between enlargement of these organs and the presence of edema." By experimenting with different varieties of milled rices it was found that the length of time during which the birds withstand the avitaminosis determines whether or not edema will occur. With rice still having some of the husk attached, the animals lasted much longer than when the rice was very highly polished and they showed edema much more frequently. It is because of these differences that the cases of polyneuritis may be either of the "wet" or "dry" variety. It was also found that young and growing birds were more prone to edema than adults and that the adrenal glands were always enlarged when it occurred. Sometimes, however, especially in adult birds, great enlargement of the adrenals was found without any edema. The author states "that the average epinephrine content per gram of gland is approximately the same as in health in cases of dry polyneuritis, and rather less than in health in the greatly enlarged adrenals from cases of wet polyneuritis." When calculated as total amount of epinephrine, per kilo of body weight, however, there was in all cases of wet polyneuritis an excess of epinephrine over the amount in health from which fact the conclusion is drawn that "a very intimate relation exists between the presence of edema and an abnormally high content of epinephrine in the adrenal medulla, when associated with malnutrition of the tissues." Addition of butter and onions to the rice reduced the incidence of edema, but in those cases in which it occurred the epinephrine content of the gland was not increased, which makes it appear that "the protection against serous effusions afforded by the butter. . . . was associated with an epinephrine-content of the adrenal glands which was not proportionate to their increase in size and was therefore within the limits of health." Dispersal of the lipoids from the cortex, as well as an increased epinephrine-content of the medulla, was found to be associated with edema production. Apparently, therefore, the vitamin A contained in butter affords a certain degree of protection against edema in pigeons fed on autoclaved rice. The influence of a deficiency of protein in the diet is not ignored as another causative factor of edema.

With regard to changes in other glands, the testicles showed an extreme

degree of atrophy, especially in pigeons, in which it is one of the most specific of the effects of avitaminosis. It is concluded that absence of vitamin B is mainly responsible for the atrophy. The atrophy affects both spermatogenic and interstitial cells. In monkeys the testicular atrophy was much less marked. In both groups of animals the ovaries also showed a certain degree of atrophy and there was decrease in the size of the uterus. Extreme atrophy of the thymus in (pigeons) on absence of vitamin B and thickening of the capsule and arteries of the spleen along with diminution of the splenic pulp in pigeons deprived of vitamin B and guinea pigs deprived of vitamin C were also noted.

With regard to other parts of the body, changes were found in the heart (atrophy) and blood vessels, but little in the kidneys. The mucosa of the urinary bladder showed marked congestive and degenerative changes in guinea pigs fed on scorbutic diets, thus affording an explanation of the comparatively frequent occurrence of hematuria in human scurvy. The weight, and presumably therefore the bulk, of the brain was increased when the food was deficient in vitamin B and in proteins and contained excess of fats and carbohydrates. This observation has an application in the treatment of headache and mental disorder. From its usual name, polyneuritis, the condition brought about in pigeons by deprivation of vitamin B might be expected to exhibit marked degenerative changes in the peripheral nerves, but such was not found to be the case. Degenerated fibers were seen but rarely in sufficient numbers to cause permanent paralysis. Death of nerve cells is the exception not the rule and the paralytic symptoms are mainly due to functional impairment of the nerve cells. This explains why recovery in such cases is common when vitamins are added to the inefficient dietary.

The last part of the monograph is devoted to the practical application of the work described in earlier parts. It is pointed out that "the health of the gastrointestinal tract is dependent on the adequate provision of vitamins of every class and in the maintenance of this, vitamin C plays a prominent part." Dysentery is shown to be favored by such food deficiency and it is pointed out that the invalid foods used in its treatment are often dangerously deficient in vitamins. Gastrointestinal disturbances of a more chronic type such as dyspepsia, mucous disease, colitis, chronic intestinal stasis and even gastric and duodenal ulcer and intussusception are also shown to bear relationships to dietetic faults. At the same time the importance of recognizing the operation of other causative factors in each of these cases is emphasized. It is the author's opinion that pellagra may result either from deficiency of protein supply, "or from deficient protein assimilation consequent on vitamin insufficiency or from a combination of both these causes." An interesting chapter is devoted to the etiology of beriberi where it is shown that avitaminosis is not the only possible cause of the disease, infection (by *B. suipestifer*) being also capable in itself of inducing it. An interesting table is given in which the postmortem appearances of polyneuritis columbarum and human beriberi are contrasted and it is suggested that there we have really to deal with two

disease entities. In a final chapter, entitled the selection of food, are given some interesting experiences in the regulation of the dietaries of patients suffering from various distressing symptoms of general ill health. The examples given emphasize that it is not only in the dietaries of the poor that dangerous deficiencies may occur, but also in those of the well-to-do. The work is one that should prove of great value to every practitioner of the healing art.

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—J. J. R. M.

# *The Journal of Laboratory and Clinical Medicine*

VOL. VII.

ST. LOUIS, NOVEMBER, 1921

NO. 2

## ORIGINAL ARTICLES

### FURTHER OBSERVATIONS ON THE PHARMACOLOGY OF BENZYL COMPOUNDS\*

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IN a preliminary article published by us<sup>1</sup> it was shown that benzyl benzoate, as well as benzyl cinnamate, are capable of producing a relaxation, in situ, of longitudinal muscles of the intestine in dogs and cats. The records in our previous paper were obtained by the Jackson method. The graphic records in our present article, dealing with the action of other benzyl compounds on the intestinal movements, in situ, were obtained, with one exception, by using the Trendelenburg method as illustrated by McGuigan.<sup>2</sup> This author makes note of three classes of movements of the intestine, namely, the pendulum movement, the peristaltic movement, and the rolling movement. In using the Trendelenburg method, these various movements can be observed through the upright glass cylinder. We shall call attention to these movements later in this paper.

Since we have already referred, in our previous article, to the research work published on this subject, we need here only call attention to a more recent and very interesting communication by Volwiler and Vliet<sup>3</sup> dealing with the preparation of various benzyl esters and their comparative rate of hydrolysis. This work is important and it led us to attempt to determine whether the relative power of benzyl esters to relax smooth muscles is proportionate to the rate at which they are hydrolyzed into benzyl alcohol. Macht has observed<sup>4</sup> that the benzyl compounds are metabolized into hippuric acid and that this change takes place more rapidly in rabbits than in dogs. He has also shown, and we have confirmed his finding, that the benzyl alcohol produces a fall in blood pressure and a relaxation of smooth muscles similar to the benzyl esters. We have observed that, while these reactions take place in the dog almost immediately upon intravenous administration of benzyl alcohol,

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they appear more gradually upon intravenous administration of benzyl esters. This would indicate that the action of benzyl esters is partly or mainly due to their gradual hydrolyzation into benzyl alcohol. The benzyl alcohol after exercising its effect combines with glycocholic acid and is excreted as hippuric acid. The action of benzyl esters upon the intestine, in situ, of dogs may be greatly hastened by dissolving the benzyl esters in alcoholic sodium hydroxide immediately before injection.

In the course of our experiments on the blood pressure, respiration, and

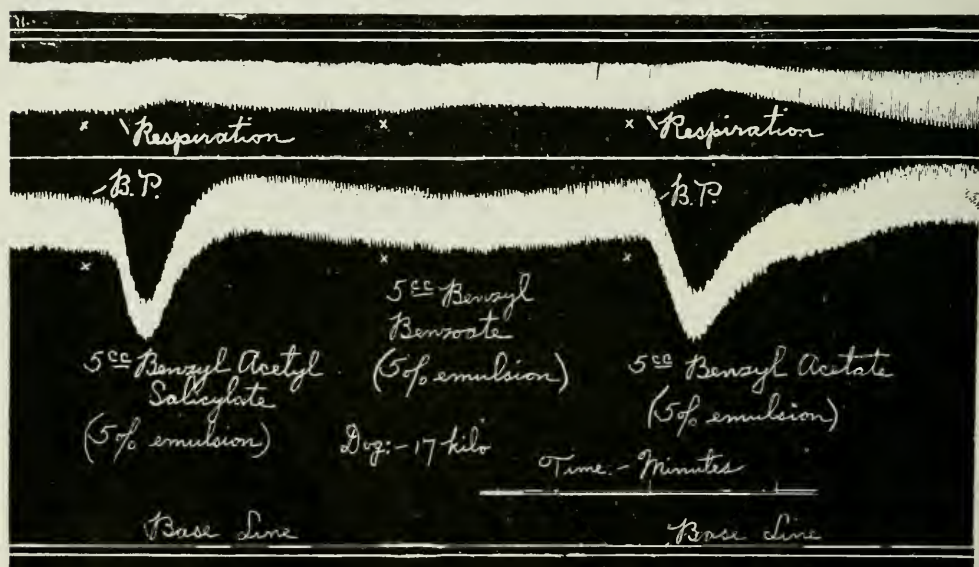


Fig. 1.—Showing the effect of benzyl ester emulsions on blood pressure and respiration.

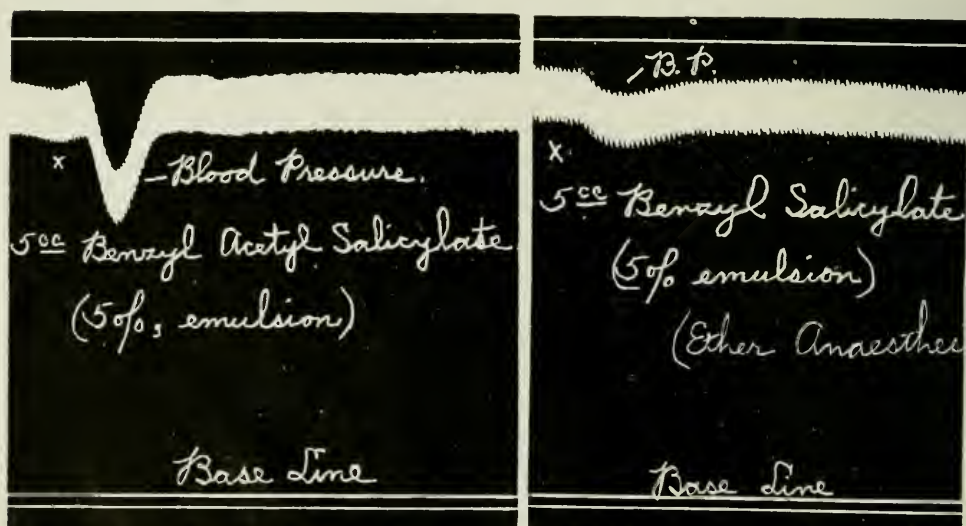


Fig. 2.—Showing the comparative action of benzyl acetylsalicylate and benzyl salicylate emulsions on the blood pressure of a dog.



intestinal movements, we have therefore attempted to throw some light on this subject.

In view of the desirability of presenting a therapeutically active benzyl ester in tablet form, various solid benzyl esters were also tested. We have studied a total of twelve benzyl esters, five liquid and seven solid, at room temperature. Due to lack of space, we shall confine ourselves to some of the results obtained from a limited number of these and publish our work on the others at a later date.

All the benzyl compounds we have investigated produce a fall in blood pressure when injected intravenously. The respiration was also depressed by relatively large doses, and in many cases we have noted an actual stoppage of respiratory movements while the heart would continue to function. Fig.

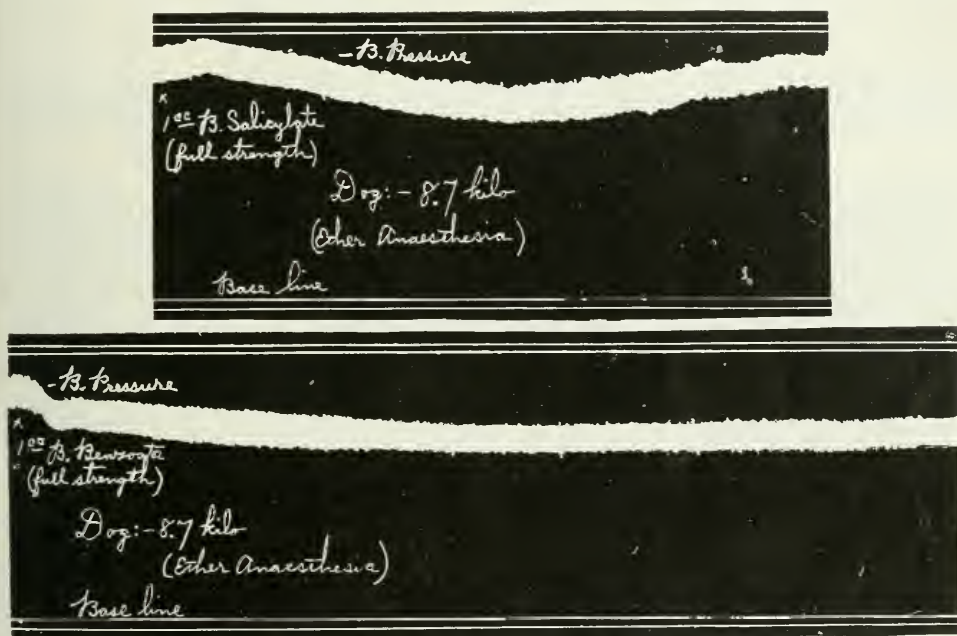


Fig. 3.—Showing the comparative action of undiluted benzyl esters on the blood pressure.

1 is an example of the action of some of the benzyl esters on blood pressure and respiration. Benzyl acetylsalicylate and benzyl acetate produce a much more marked effect upon blood pressure and respiration than benzyl benzoate in the same dose. Benzyl acetate shows a more pronounced reaction on respiration. Fig. 2 shows the action of benzyl salicylate as compared to benzyl acetylsalicylate in another dog. Benzyl salicylate produced only a very slight fall as compared to that produced by the same dose of benzyl acetylsalicylate. Fig. 3 demonstrates the comparative action of the salicylate and the benzoate on the blood pressure when injected undiluted. While these blood pressure records suggest a theory of action proportionate to hydrolysis, our experiments on the intestine, in situ, show that, on that organ at least, two of the esters, benzyl salicylate and benzyl acetylsalicylate, do not behave according to that

theory. Fig. 4 is interesting because it demonstrates the gradual and prolonged fall in blood pressure caused by the injection of benzyl acetylsalicylate in sweet almond oil, as compared to the abrupt and short fall in blood pressure produced by a relatively smaller dose in the form of an emulsion. This difference would naturally be expected because the oil delays the hydrolysis.

During the first period of our investigation on the action of benzyl compounds upon intestinal movements, we injected the esters in the form of 5 per cent emulsions. This method was quite satisfactory as long as we had to do with liquid esters alone; but, in order to investigate and compare the action of the entire series, including esters solid above body temperature, it became necessary to find a solvent suitable for all of them. We dissolved, therefore, the benzyl esters in sweet almond oil (1:5) and injected these solutions at a temperature slightly above that of the body; that is, approximately at 39° to 40°C. The esters with a higher melting point remained in solution

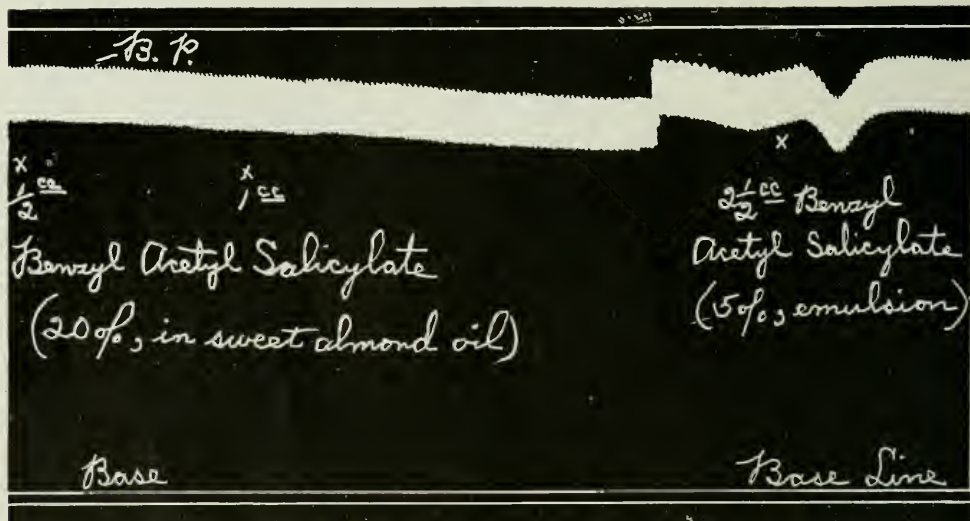


Fig. 4.—Showing the difference in effect of benzyl acetylsalicylate, in an oily solution and the same ester in an emulsion form, on the blood pressure of a dog.

in this proportion sufficiently long to be distributed and absorbed. These oily injections produced a very slow and gradual intestinal relaxation in the dog while the cat's intestine responded almost immediately to them (Figs. 5, 6, 7 and 14). The same difference in response was noted when the undiluted liquid esters were injected. The cat evidently metabolizes the benzyl compounds much more rapidly than does the dog; the cat, therefore, seems to be an exception to the rule of the difference in benzoic acid metabolism between herbivora and carnivora, as mentioned by Macht.<sup>4</sup> When an oily solution of benzyl fumarate was injected into a dog, the gradual intestinal relaxation and the gradual fall in blood pressure proceeded at the same rate (Fig. 5). The drum was stopped for a short period of time at *a*.

Fig. 6 is a tracing showing the action of benzyl fumarate and benzyl acetylsalicylate in oily solutions upon the intestine, in situ, of a cat. In this

particular experiment, the so-called rolling movements of the intestine were noted. Benzyl fumarate, 0.5 c.c. of a 20 per cent solution in almond oil, partially stopped the rolling movements, whereas the same amount of a 20 per cent benzyl acetylsalicylate solution in almond oil virtually paralyzed the intestinal segment. The increased intestinal movements which followed the

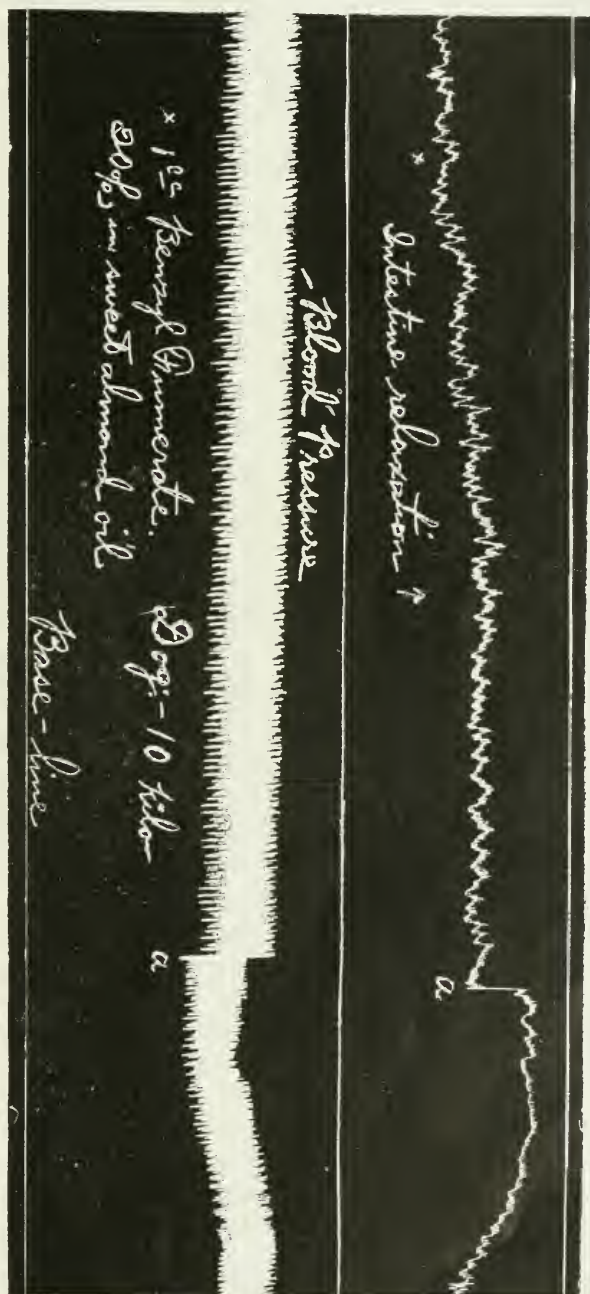


Fig. 5.—Showing the effect of a benzyl fumarate solution, in sweet almond oil, on the blood pressure and the intestinal movements. The Jackson method of recording intestinal movements was used in this experiment.



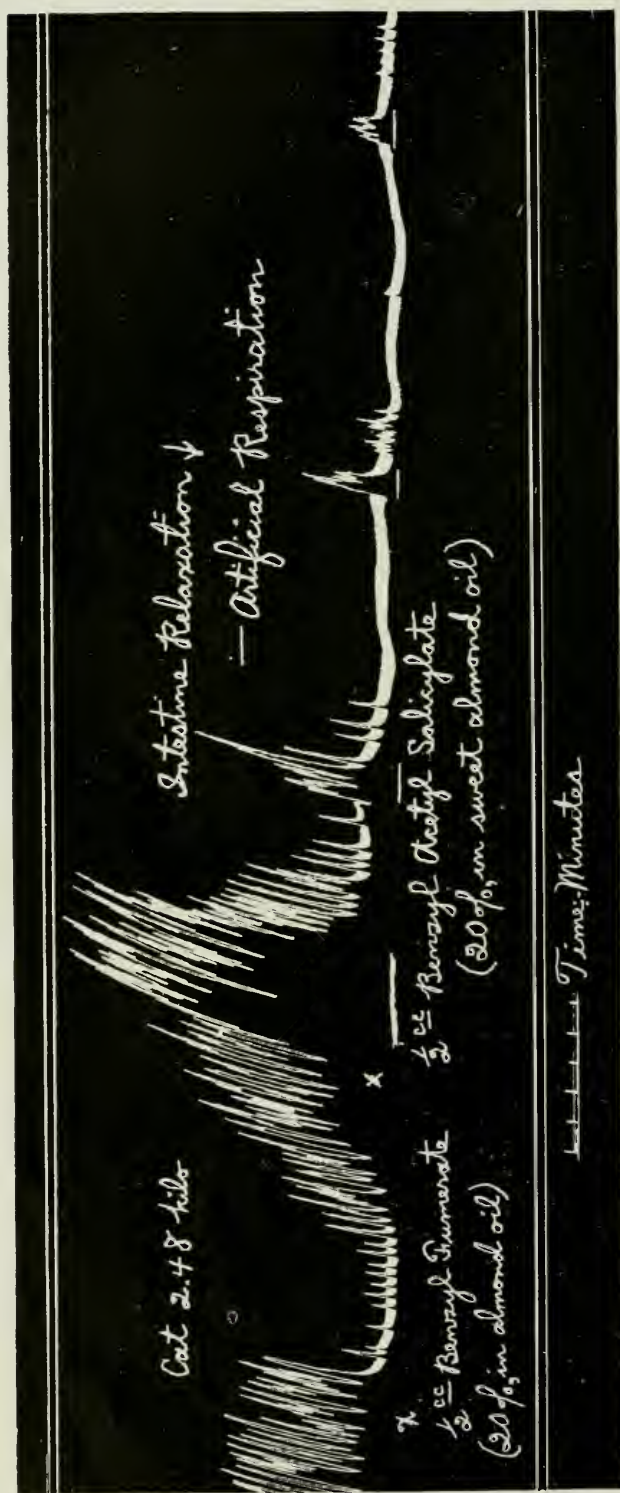


Fig. 6.—Showing the comparative action of benzyl fumarate and benzyl acetylsalicylate on the intestine of a cat.

injection of benzyl acetylsalicylate in this instance were undoubtedly due to a partial asphyxia induced by stoppage of respiration. As the respiration was reestablished, it will be noted that the intestinal relaxation continued. Control experiments were carried out to show any possible influence of sweet almond oil (Fig. 7). This oil had only a negligible effect—if any—upon the movements of the intestine, while 1 c.c. of a 20 per cent benzyl fumarate solution in sweet almond oil produced a distinct relaxation of the intestine in the same cat. This striking difference in response to intravenous benzyl ester injections between the dog and the cat led us to search for a means of hastening the hydrolysis and thereby the action of the benzyl esters in the dog.

This was accomplished by dissolving the benzyl esters in alcoholic sodium hydroxide immediately prior to the injections (90 seconds). It was thought

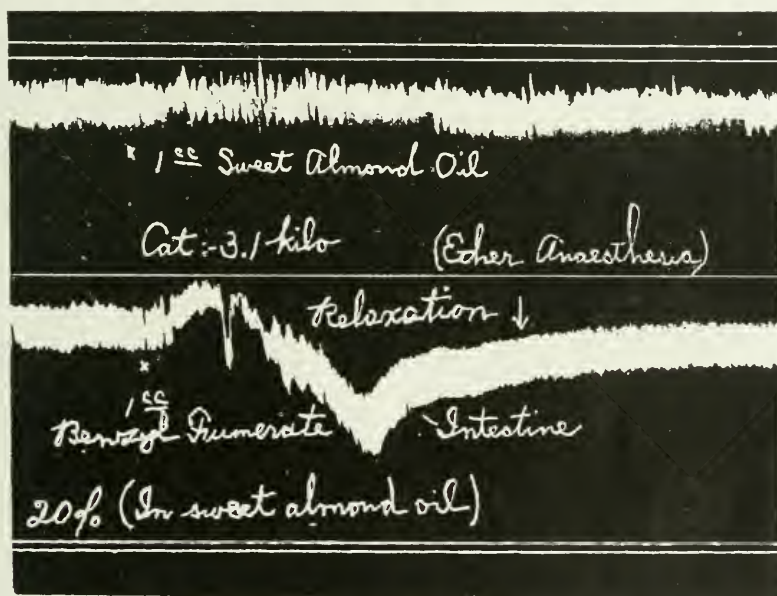


Fig. 7.—Showing the comparative action of sweet almond oil and a benzyl fumarate solution in sweet almond oil on the intestine of a cat.

that, since the hydrolysis of the benzyl esters injected into the dog's blood stream proceeded very slowly, an addition of alkali in this manner would start the hydrolysis and bring about a reaction on the intestinal organ similar to that which would result from oral administration of these esters. We proceeded, therefore, (Fig. 8) by injecting 1 c.c. of  $n/10$  NaOH in 95 per cent alcohol into the vein of a dog prepared for this experiment, and recorded marked intestinal contractions. This was followed by an injection of 1 c.c. of a 20 per cent solution of benzyl acetylsalicylate in  $n/10$  alcoholic NaOH. This produced a marked intestinal relaxation. After a considerable lapse of time (approximately an hour and a half) the same experiment was repeated substituting benzyl fumarate for benzyl acetylsalicylate (Fig. 9). The records show that the benzyl acetylsalicylate, which contains only slightly more than



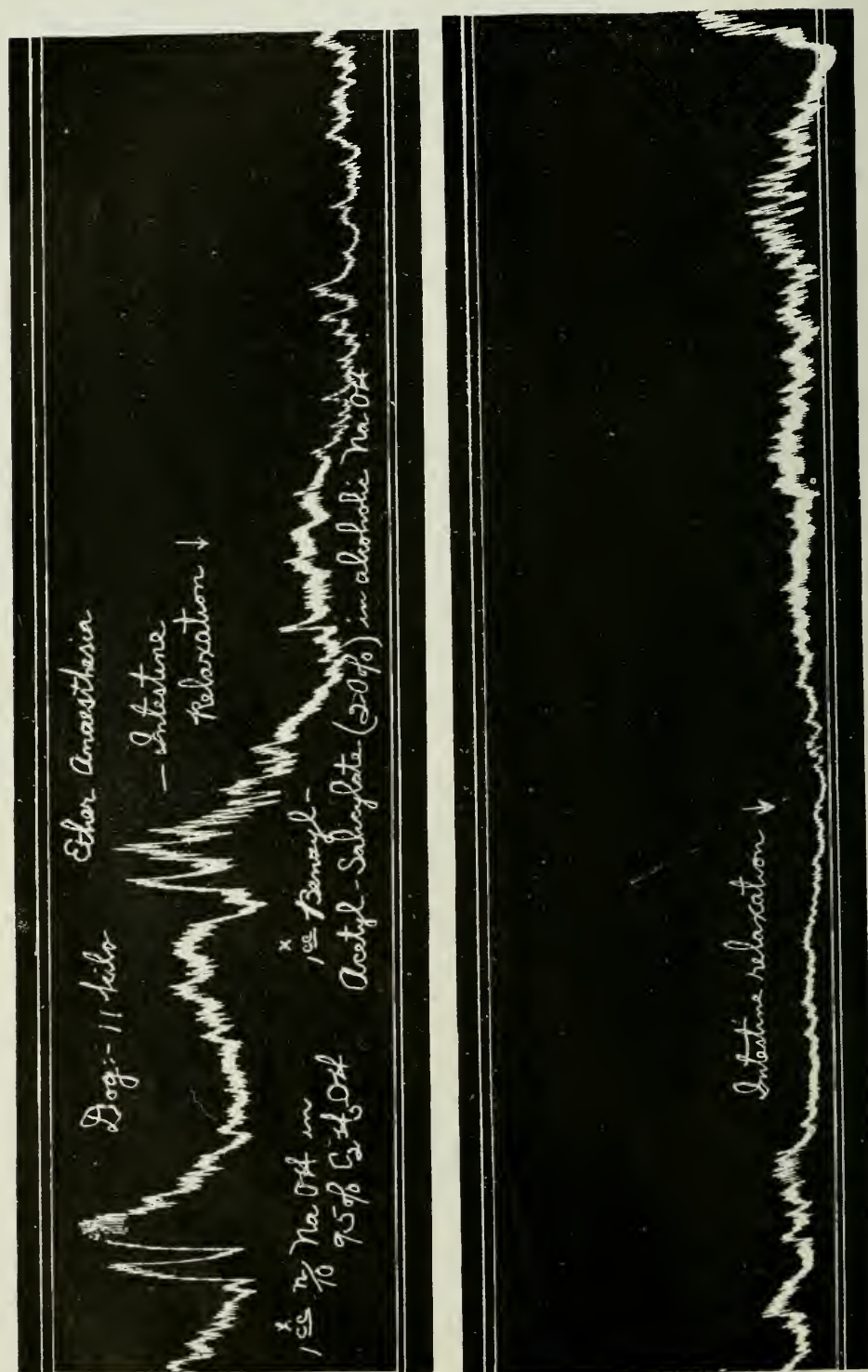


Fig. 8.—For description see text.

half as much benzyl as the same dose of benzyl fumarate, produced a more marked and more lasting intestinal relaxation in the same dog.

Fig. 10 shows the result of the intravenous injection of a 20 per cent solution of benzyl acetylsalicylate in  $n/10$  sodium hydroxide, prepared approximately half an hour prior to the injection. The reaction was almost immediate and very marked.

Similar experiments were made with benzyl benzoate (Fig. 11). An intravenous injection was made into a dog, of 1/2 c.c. of benzyl benzoate, undiluted. As usual, there was hardly any reaction on the dog's intestine from this injection. This was now followed by 1/2 c.c. of benzyl benzoate dissolved in

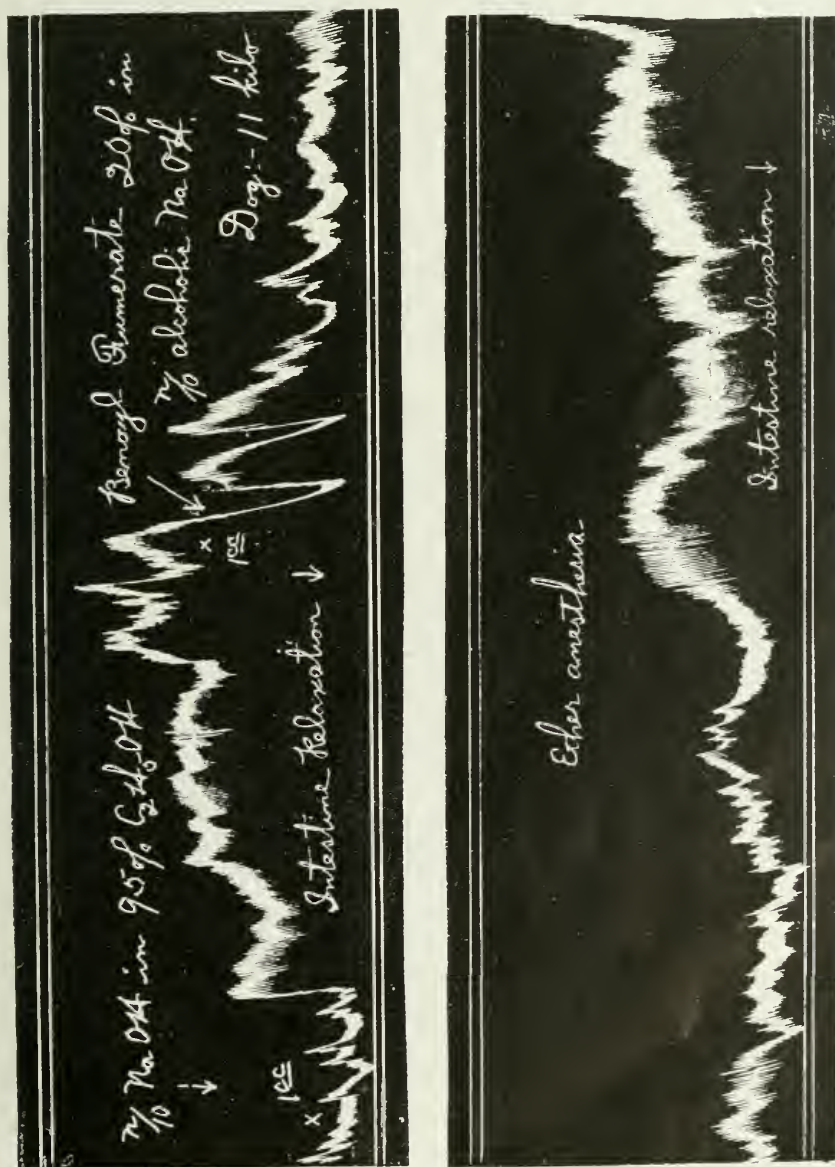


Fig. 9.—For description see text.

2-1/2 c.c. of n/4 alcoholic sodium hydroxide. A marked intestinal relaxation followed almost immediately. After the return of the intestine to normal, 2-1/2 c.c. of n/4 alcoholic sodium hydroxide alone was injected. The result was a strong contraction of the intestine. In order to verify our point, we next repeated the injection of 1/2 c.c. of benzyl benzoate in 2-1/2 c.c. of n/4 alcoholic sodium hydrate, and again we obtained a marked relaxation. The intestine was then again allowed to return to normal, after which an injection



Fig. 10.—For description see text.

of benzyl acetylsalicylate was made in a quantity containing the same amount of benzyl as 1/2 c.c. of benzyl benzoate, that is, 0.66 c.c. dissolved in 2-1/2 c.c. of n/4 alcoholic sodium hydroxide. The intestinal relaxation obtained from this dose was very rapid and pronounced and stronger than that produced by the proportionate dose of benzyl benzoate. The record in this instance does not do full justice to the power of benzyl acetylsalicylate to relax the intestine, because the respiration ceased, and as a result of the asphyxia, the intestine started to



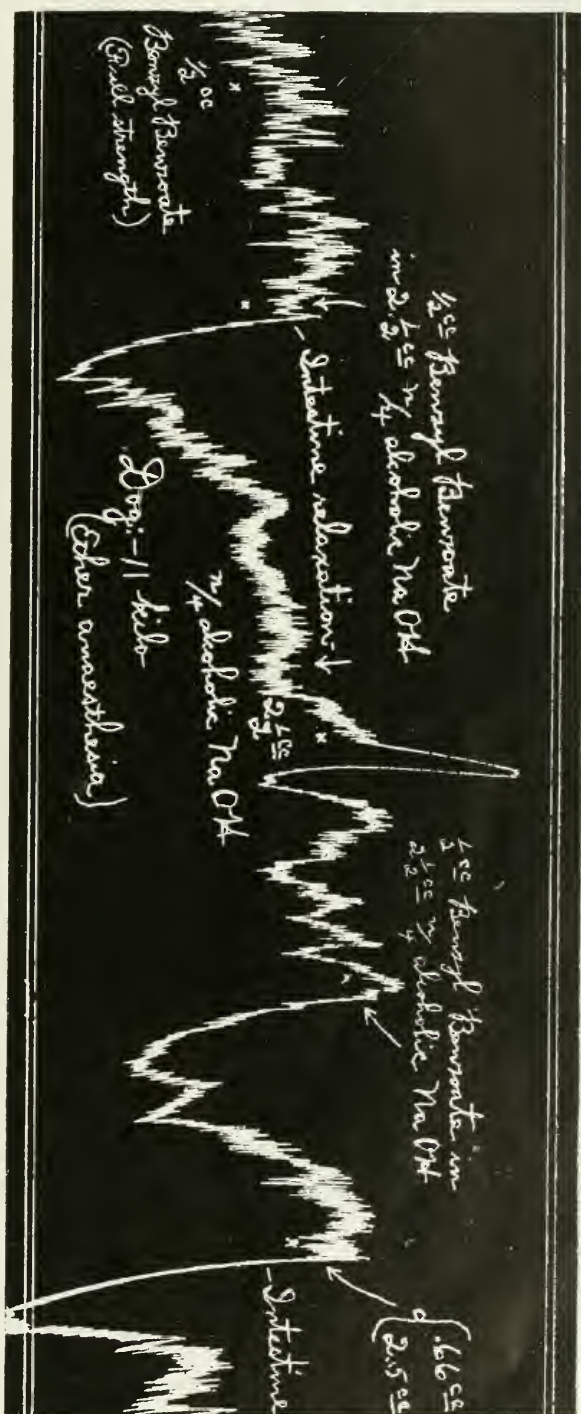


Fig. 11.—For description see text.

contract again too soon. It should be noted that the amounts of sodium hydroxide used in these experiments were only  $1/10$  to  $1/4$  of those required to hydrolyze the total amounts of benzyl present in the injections.

The experiments with alcoholic sodium hydrate were intended only to throw further light upon the fate in the body and the manner of action of the benzyl esters.

The strikingly powerful action, upon intestinal muscles, of benzyl salicylate and benzyl acetylsalicylate (Figs. 6, 8 and 14) led us to experiment further with them. The salicylate, according to Volwiler and Vliet, hydrolyzes more slowly, *in vitro*, than any of the esters investigated by us, yet its power of relaxing the intestine is so great as to be out of all proportions to its rate of hydrolysis. The benzyl acetylsalicylate is still more powerful; in fact, it surpasses all the esters investigated.

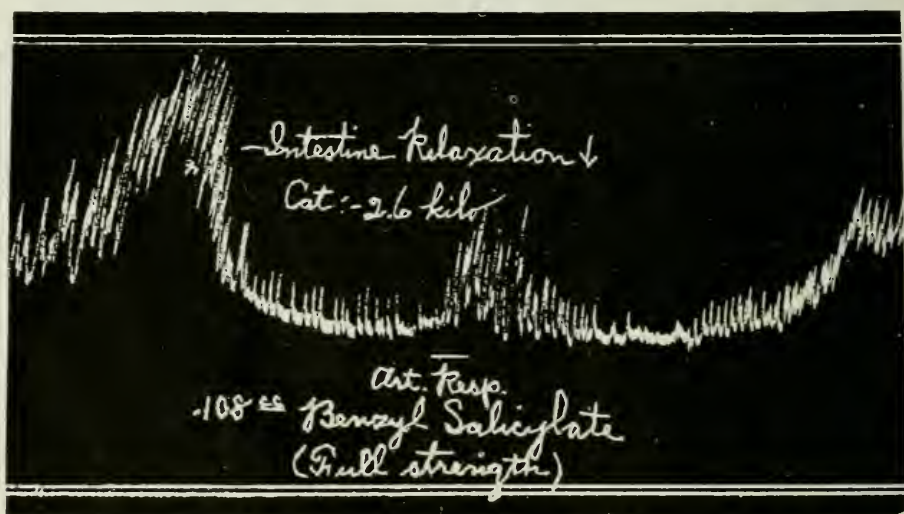


Fig. 12.—Showing the action of benzyl salicylate on a cat's intestine, when injected undiluted.

Fig. 12 shows the effect of an intravenous injection of 0.108 c.c. benzyl salicylate, undiluted, into a cat (corresponding in benzyl content to 0.1 c.c. of benzyl benzoate). The same amount of benzyl salicylate dissolved in 0.2 c.c. of oil produced a much more pronounced and prolonged relaxation of the intestine in a cat than the molecular amount of benzyl in the form of benzyl benzoate (0.1 c.c. plus 0.2 c.c. oil) (Fig. 13). It is evident, therefore, that benzyl salicylate owes its action on the intestine to something else besides its benzyl content and its rate of hydrolysis. This may be true for the benzyl acetylsalicylate also. This ester is closely related to the salicylate and its high rate of hydrolysis, *in vitro*, does not cause a rapid formation of benzyl alcohol, but rather a splitting off of the acetyl group, followed by a gradual hydrolysis of the remaining benzyl salicylate. Volwiler and Vliet's experiments indicate that the velocity of saponification of the benzyl group in benzyl acetylsalicylate is the same as that in benzyl salicylate.

The pharmacologic aspect of this question is being further investigated





Fig. 13.—Showing the comparative action of benzyl benzoate and benzyl salicylate on the intestine of a cat, when injected in oily solutions in benzyl-molecular amounts (0.2 c.c. of a mixture of 1 gm. benzyl benzoate and 2 gm. oil, and 0.2 c.c. of a mixture of 1.08 gm. benzyl salicylate and 2 gm. oil).

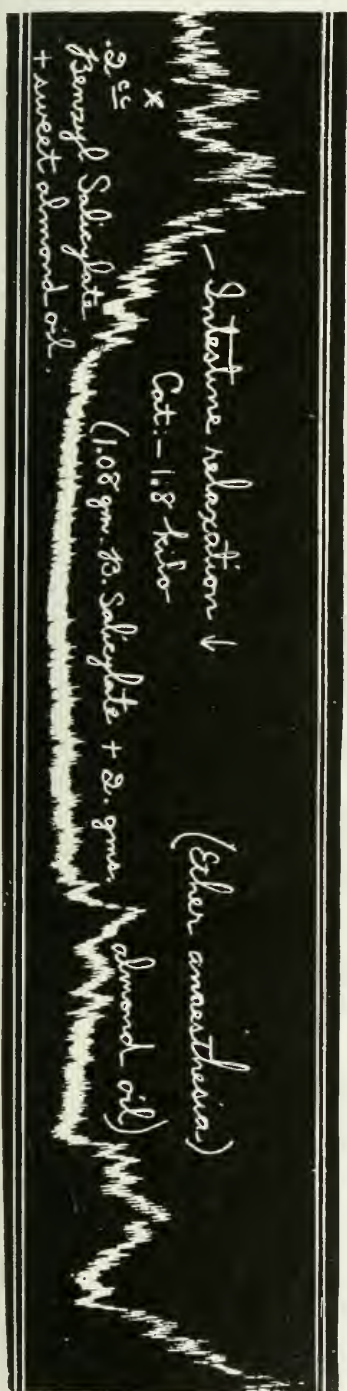
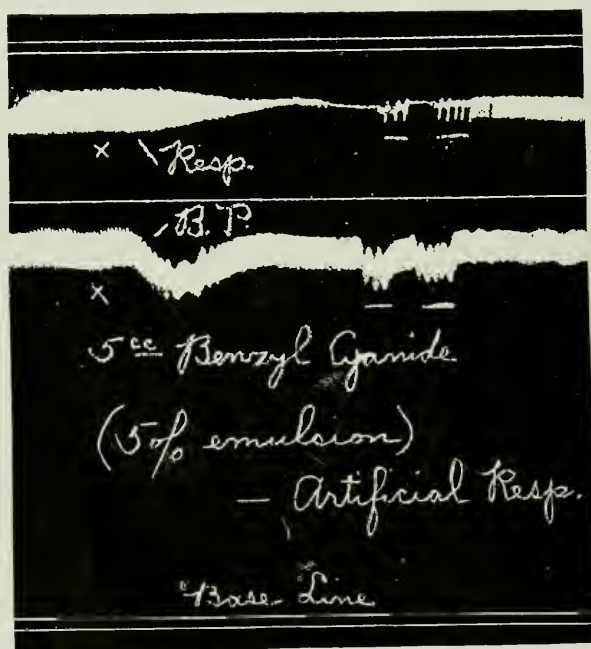


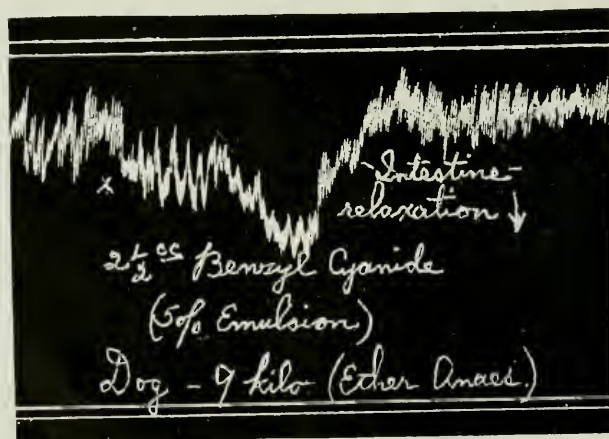
Fig. 14.—Showing the action of benzyl salicylate on a cat's intestine, when injected in an oily solution.

by us. We may say at this time that, from the numerous records we have produced, in addition to those published here, there seems to be a correlation of rate of benzyl hydrolysis and smooth-muscle-relaxing property in the series of benzyl esters investigated by us, with the exception of the salicylate and acetylsalicylate.

In addition to the foregoing data, we present a few tracings showing the action of the blood pressure, respiration and intestinal movements, produced by benzyl cyanide and that on the intestine by benzyl phenolate (Figs. 15 and 16). It will be seen that these benzyl compounds also produce relaxation of



A



B

Fig. 15.—Showing the effect of a 5 per cent benzyl cyanide emulsion on the blood pressure, respiration and intestinal movements in a dog.

the intestine and that benzyl cyanide is similar to other benzyl esters in its action on blood pressure and respiration. The benzyl phenolate record shows that one may look for intestinal relaxants in the domain of benzyl ethers also.

#### CONCLUSIONS

All the benzyl esters we have investigated produced relaxation of intestinal muscles, lowered the blood pressure and depressed the respiration in a manner corresponding, in a general way, to the action of benzyl benzoate as demonstrated by Macht.

The power to relax smooth muscles and thereby relieve spasmodic pain, seems, in the cases of simple benzyl esters, to be proportional to their rates of

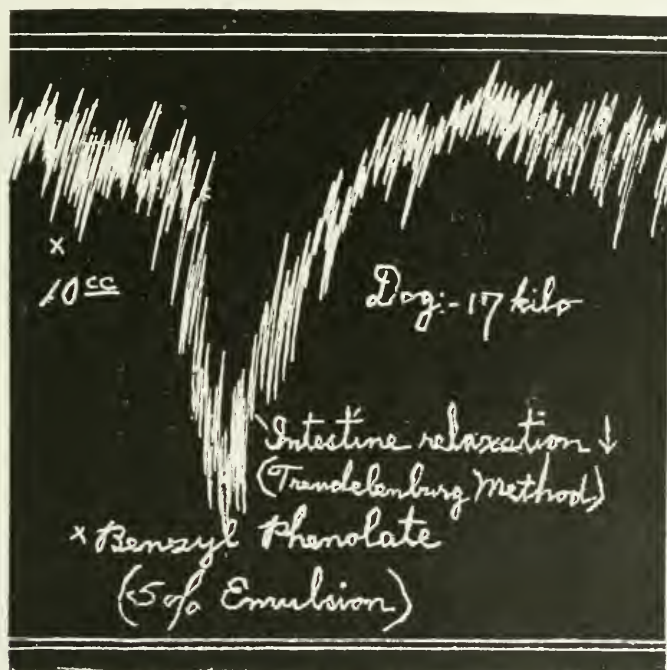


Fig. 16.—Showing the effect of a 5 per cent benzyl phenolate emulsion on a dog's intestine.

hydrolysis; but in the cases of benzyl esters containing substituting groups such as in benzyl salicylate and benzyl acetylsalicylate, rate of hydrolysis to give benzyl alcohol is not a criterion of pharmacologic action.

Of the twelve benzyl compounds we have tested, benzyl acetylsalicylate appears to be the most powerful intestinal relaxant. Benzyl salicylate is also very efficient. Further investigation of these esters is now being carried out. A summary of our experiments with the esters of higher melting points will also be published later.

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## A TECHNIC FOR MAKING A BILIARY FISTULA

BY F. C. MANN, M.D., MAYO FOUNDATION, ROCHESTER, MINNESOTA

OF THE many methods described for making biliary fistulas one of the following procedures has been generally employed: (1) Suturing the gall bladder to the abdominal wall and opening it so that bile drains to the exterior; (2) suturing a cannula or rubber tube in the gall bladder; (3) excising the common bile duct at the point of entrance to the duodenum with a piece of duodenal wall and suturing it to the skin; and (4) placing a cannula in the common bile duct.

All of these methods have been tried in our laboratory and have been found unsatisfactory in many ways. The most successful of these methods of making a biliary fistula we have found to be suturing the gall bladder to the skin. In many experiments the bile will drain through the gall bladder for a long time. Usually, however, the viscus becomes infected, which may seriously interfere with the results of the experiment. It should be noted that when the bile is drained through the gall bladder, it is absolutely essential that the common bile duct is doubly ligated and sectioned. Even after such a procedure the partial re-establishment of bile drainage into the intestine is not uncommon. When cannulas are used, they quite frequently ulcerate out or the area surrounding them becomes infected, making the work and results unsatisfactory. The transplantation of the duct with a piece of the duodenal wall is often a failure because of the poor blood supply to the transplanted tissue, because of the tension necessarily placed on the sutures and tissue which hold the duct stretched to the abdominal wall, and because of injury to the pancreas and infection. In order to overcome some of these difficulties, another method was devised for the work in our laboratory and has proved very satisfactory. We have used it in the dog only, but it could be employed in other species such as the cat and goat. The essentials of the technic are, first, definite fixation of the common bile duct in a superficial position before its relation to the duodenum is disturbed and, second, draining the duct to the exterior. The operative procedure is carried out in two stages. In the first stage the duodenum is immobilized just under the skin in a manner so that the common bile duct is close to the surface. After complete healing has taken place, the duct is exposed, the distal end ligated, and the proximal end left open, flush with the skin.

Briefly the steps of the operation are as follows: Under ether anesthesia and with sterile technic a midline or right rectus incision is made. The incision should be carried forward as far as possible without opening the pleural cavity. The pylorus and first portion of the duodenum are located and pulled up into the wound. A small opening through the mesentery of the duodenum is made about 4 cm. on each side of the point of entrance of the common bile

duct into the duodenum and between the duodenal wall and the pancreas. Care must be taken in making these openings not to injure the pancreas and the blood vessels going from the pancreas to the duodenum, and in making the lower opening not to injure the major pancreatic duct. Through these openings the peritoneum and, later, the fascia are sutured with double No. 2 chromic catgut. This procedure leaves the duct-bearing portion of the duodenum just under the skin (Fig. 1). The superficial fascia and the skin are then sutured over the transposed loop of intestine (Fig. 2). In closing around and over the upper and lower ends of the loop care must be taken not to cause too much pressure as obstruction may be produced. After complete healing has taken place, which requires from seven to fourteen days, at a second operation a small incision is made over the point of the transposed loop

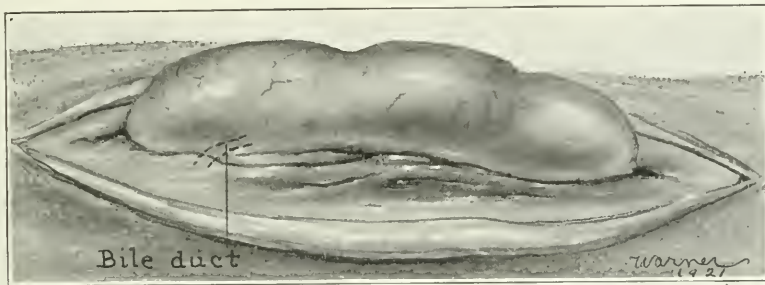


Fig. 1.—The first part of the duodenum pulled out into the wound and the peritoneum and fascia sutured through the openings made in the mesentery, thus fixing it firmly in position with the bile duct partially exposed.

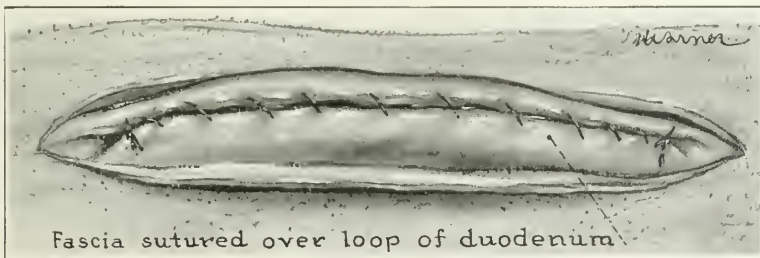


Fig. 2.—The subcutaneous fascia sutured over the transposed loop of the duodenum.

where the common bile duct is located, the duct is exposed, ligated, and opened so that it will drain at its point of emergence from the skin. It is usually a good plan to keep a soft rubber catheter in the duct for the first day or two after operation. This prevents inflammation following operative trauma from obstructing the flow of bile. The operative wound usually heals quickly and in a very few days after the second operation observations can be started.

We have found this method of making a biliary fistula more successful and satisfactory than any other. By simply passing a catheter into the opened end of the duct observations can be taken on the secretory activity of the liver, the secretory pressure of the organ, or the movements of the gall bladder. We have made repeated observations on some of our animals for periods of six months or more, during which time the animal was maintained in an appar-



ently normal condition. However, since bile seems to be an absolutely necessary secretion for the maintenance of life and good health, sooner or later all our animals have developed serious conditions regardless of the method of making a bile fistula or the method of feeding. As in all fistulas there is a tendency toward a healing over of the opening, for this reason it is desirable to catheterize quite frequently. The cicatrization is not so troublesome, however, as when the bile is drained through the gall bladder and after the first few weeks catheterization is not necessary.

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## A TECHNIC FOR THE ESTABLISHMENT OF A PERMANENT PANCREATIC FISTULA WITH THE SECRETION OF INACTIVE PROTEOLYTIC FERMENT\*

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**F**EW methods which have been used for the establishment of permanent pancreatic fistulas in animals have been entirely successful, and none ideal.

Claude Bernard tied a glass tube into the pancreatic duct and led it through the abdominal wall. Ludwig's followers fastened into the duct in similar manner a T-shaped piece of twisted lead wire. These procedures gave fistulas which remained patent for a few days only, the glass tube and the wire coming out and the duct cicatrizing. Yet these were steps forward since they permitted the maintenance of a fistulous tract for a time sufficient to allow the inhibitory effects of narcotization and operative trauma to wear off, although infection almost always developed immediately and produced incessant irritation of the gland and stimulated it to continuous secretion regardless of whether or not digestion was in progress.

Pavlov, in 1879, and Heidenhain, in 1880, independently published methods which were very much alike. Pavlov's technic has become classic and has been employed almost universally. It is as follows:

The duodenum is delivered through a midline or right rectus incision, the entrance of the pancreatic duct isolated, and a rhomboidal piece of the duodenal wall bearing the orifice of the duct is its center, is excised. The opening in the bowel is closed and the isolated piece of intestine sewed into the slit in the abdominal wall; the duodenum itself is retained against the anterior parietes by temporary suspension sutures. The drawbacks to this procedure are: An active proteolytic juice is secreted which erodes the abdominal wall, causes pain and bleeding, and reflexly inhibits secretion; the pancreatic juice is rendered impure by the secretion of the flap of transplanted duodenum; the amount of enterokinase activating the trypsinogen is not under quantitative control; tension is put on the duct by its traversing of the entire thickness of the abdominal wall, and by the insecure fastening of the duode-

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\*The study was made by the Division of Experimental Surgery and Pathology, Mayo Foundation, under the direction of Dr. F. C. Mann.

num; these circumstances often lead either to retraction or to necrosis of the papilla.

Babkin modified Pavlov's technic in order to avoid the secretion of an active proteolytic juice. After the primary wound had healed, he dissected off the oval piece of mucous membrane from around the orifice of the fistula. However, such a duct so treated is likely to become strictured and is kept open with difficulty.

Theoretically the ideal method would permit emptying the juice into the intestine during the intervals between experimental collections. In an effort to attain this Fodera, in 1894, succeeded in getting a special T-shaped metallic cannula to heal into the duct so that the juice could be collected exteriorly or allowed to run into the intestine as desired; but by this procedure Fodera could have no assurance that juice might not also be emptying into the intestine as well as externally.

Lattes, in 1912, developed a technic largely similar to that of Pavlov; it differed in that the primary incision was made through the right mammary line, the duodenal papilla alone was excised, and the duct brought through the muscular layers of the abdomen in such a fashion that contraction of these would keep it collapsed save when a cannula was introduced. Thus a continent fistula was obtained. Lattes found that an ampulla was formed just back of the obstruction, that the duct lengthened, and that in compensation the other pancreatic duct became longer.

Frouin, in 1913, evolved a method for the formation of a fistula to secrete inactive juice. Through an incision somewhat to the right of the midline the duodenum was brought up and the major pancreatic duct cut at its juncture with the gut. The duodenum was then sutured into the opening in the muscle, forming a tampon, and the duct brought through a stab wound in the skin about 2 cm. from the primary incision. Two complications were encountered: intestinal obstruction and retraction and obliteration of the orifice with marked dilatation of the canal back of the obstruction if the duct were everted insufficiently.

Following the principle of transplantation of the duodenum under the skin as developed by Mann in establishing biliary fistulas (see illustrations in preceding paper) we evolved a technic for the formation of pancreatic fistulas which seems to offer certain advantages over those heretofore suggested. The procedure may be performed either as a one-stage or a two-stage operation.

*One-Stage Operation.*—A curved incision about 10 cm. long is made through the skin of the abdomen, starting just below the xiphoid process and circling to the right until at its middle the cut is approximately 2 cm. from the midline. This curved skin flap with sufficient of the underlying subcutaneous tissue to insure adequate blood supply is reflected to the linea alba and the ordinary midline laparotomy incision is made. The duodenum is located, delivered into the wound, and turned to the right. The blood vessels in the immediate neighborhood of the entrance of the major pancreatic duct into the intestine are ligated and severed, the pancreas is separated from the duodenum in this region, and the duct itself isolated. (In the dog the free part of the

duct of Santorini, which is about 2 mm. wide and 0.75 mm. long, will be found on the left side of the duodenum near the posterior part of the portion of the pancreas which is directly applied to the gut. A section of the gland overlaps and hides it, but by pushing this back at the point where a large blood vessel is noted on the surface of the bowel in this area it may be easily located<sup>12</sup>). The transplantation of the duodenum is accomplished by rotating its axis to the right, and approximating the two edges of the abdominal wall under the duodenum by means of four single mattress sutures, No. 2 chromic catgut, which include both fascia and peritoneum (two sutures to each side with the location of the duct at about the middle of the incision). Openings just sufficient for the duodenum to pass through snugly, but without constriction, are left at the upper and lower ends of the wound. In the rotation of the duodenum a small portion of the pancreas is brought out of the abdomen and thereby the entrance of the duct into the gut becomes very superficial. The mattress sutures pass through this portion of the pancreas in an oblique direction; the head of the needle is passed first in order not to injure blood vessels by the sharp point, and comes out at the juncture of the intestine and the right layer of the mesoduodenum. At each of these points a portion of the

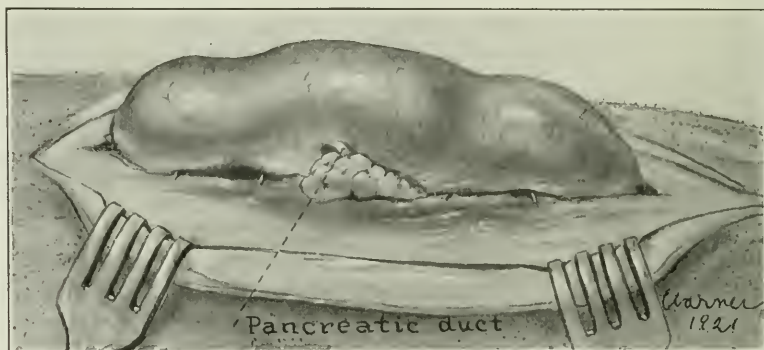


Fig. 1.—The duodenum transplanted outside the abdominal cavity with the major pancreatic duct still in situ preparatory to the establishment of the fistula.

bowel wall is included in the sutures for secure anchorage. The sutures are tied and the abdominal cavity shut off from the field of operation (Fig. 1).

The skin on the right of the initial incision is reflected in its central portion for approximately 1 cm., depending on the amount of room needed for the easy reception of the duodenum which is brought over to the right and fitted into the cavity thus made. Catgut sutures passing through the peritoneal and muscular coats of the intestine and through the superficial fascia of the abdominal wall are inserted to maintain the bowel in its new position.

The pancreatic duct is dissected free for the short distance which it runs obliquely beneath the serosa of the gut and is partially severed at the point where it enters the muscular layer. A very fine silk suture on a small needle, such as is used for blood vessel anastomosis, is passed through the free left lip of the duct, a section of a ureteral catheter is inserted into the canal for a short distance, and the duct completely severed. The serosa is brought together over the small wound in the intestinal wall by one or two Lembert



sutures. Three other fine silk strands are then passed through the extremity of the severed duct at equal distances. A small stab wound is made in the original semicircular flap of skin just over the point where the duct disappears into the pancreas and by means of the strands the duct with the catheter is brought through the opening. The original skin incision is closed by suturing the subcutaneous fascia with catgut and the skin with interrupted linen stitches. The end of the duct is everted and securely fastened to the skin by means of the four silk sutures, the catheter is left in place and its free portion allowed to run along the abdominal wall for from 4 to 5 cm. where it is fastened by two or three superficial stitches. The wounds are iodinated and covered with collodion dressings (Fig. 2).

*Two-Stage Operation.*—At the first operation the duodenum is transplanted under the skin as described for the one-stage operation. A month later (if the interval is shorter a great deal of bleeding is encountered from the new-formed vessels at the site of the old wound) at the second operation the duct is brought to the surface. If the time can be spared the two-stage

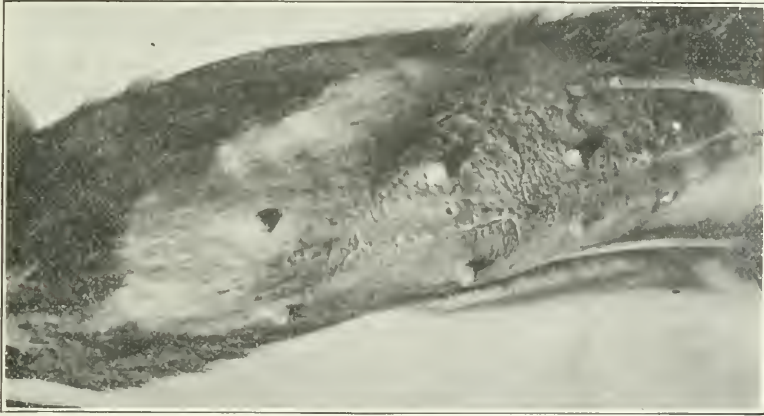


Fig. 2—E130. Photograph of dog taken twenty-three days after a one-stage operation for the establishment of a pancreatic fistula. The orifice of the pancreatic duct, bulging due to the transplanted duodenum, and the intact condition of the skin of the abdomen are shown. The animal was killed by ether three months and three days after the formation of the fistula and the pancreas grossly was found to be normal.

method is preferable, since the animal may then be in good nutritive condition before the establishment of the fistula, and the chances of peritoneal infection are lessened.

This technic suggests that intestinal obstruction may occur. We have never encountered this complication, and believe that it can be avoided easily by proper care in the transplantation of the duodenum. However, at times the common bile duct becomes slightly dilated, probably because of the rotation of the intestine, but this is of little significance.

Immediately after operation the animal is provided with a metal collar to prevent any disturbance of the wound. There is no secretion from the fistula for the first three or four days, at the end of which time the catheter comes out spontaneously. Thereupon a slight irritative secretion frequently appears and may last for a week, while the pancreatic gland secretes whether or not food is given. The secretory conditions are normal and the wound

completely heals within a fortnight, when experimental observations may be begun. As a rule, the fistula shows little tendency to close, but when the tendency is apparent, it is most evident between the tenth and fourteenth days, and may be avoided easily by passing a probe or cannula into the opening every second day.

#### COMMENT

The animals used (dogs) have been kept ordinarily on a diet of milk, with sodium bicarbonate added to make up for the loss of alkali through the juice. No untoward results, such as have been described by Pavlov, have been noted from feeding meat. The animals, save for a slight loss at the time of operation, maintain their weight, especially if the two-stage technic is employed.

The juice secreted is completely inactive so far as proteolytic ferment is concerned, and has no effect on the skin of the abdomen. For protein-digestion experimentation the juice is activated by the addition of enterokinase solution prepared by the method of Bayliss and Starling. This solution may be kept for months at room temperature. After a secretory meal has been given, the juice may be collected in many ways. We have found it most satisfactory to tie the dogs to a frame and apply a metal funnel over the duet opening. During the times of noncollection the animals lick off the abdomen so that the amount lost is not so great as would at first appear.

The success of the technic suggested depends on the following factors: the avoidance of opening the intestine; the fixation immediately under the skin of the point of exit of the duet from the pancreas; and the bringing of the duet to the surface of the abdomen through an incision other than the operative incision. These are accomplished by severing the duet just as it enters the tunica muscularis of the intestine, transplanting the duodenum under the skin with rotation of its axis to the right, and making a primary curved incision away from the point of bringing out the duet. Thus are avoided the danger of peritonitis and stricture of the duodenum, retraction and necrosis of the duet from tension, and subsequent closure of the fistular orifice by its inclusion in the operative scar, factors which are most inimical to success.

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## SOURCES OF ERROR IN DETERMINING THE RESPIRATORY QUOTIENT IN THE BABY RESPIRATION APPARATUS\*

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DURING the past year we have been making studies at the Massachusetts General Hospital of the basal metabolism and respiratory quotient of abnormal infants in the Benedict baby respiration apparatus. We have found that the respiratory quotient in pathologic cases often shows unexplainable variations despite the fact that an absolutely correct alcohol check had been obtained. It is the aim of this paper, therefore, to give in detail the possible causes of error which are likely to occur in determining the respiratory quotient. We now feel that with certain experimental conditions under control it is possible to determine the true respiratory quotient of the living subject for short periods of half an hour, and that such short observations may add significant data to pathologic physiology.

The respiratory quotient is computed by dividing the volume of carbon dioxide excreted by the volume of oxygen consumed in a measured period of time. The importance of the relationship between oxygen consumption and carbon dioxide production was first recognized by Pflüger. When the respiratory quotient is carefully determined it indicates the character of the material being burned up in the body. For instance when carbohydrate is being burned the respiratory quotient is unity, that is, for every hundred volumes of carbon dioxide excreted, there are one hundred volumes of oxygen absorbed. Thus the volume ratio  $\frac{\text{vol. CO}_2}{\text{vol. O}_2}$  is equal to a respiratory quotient of 1.00. The respiratory quotient for protein, that is,  $\frac{81 \text{ vols.}}{100 \text{ vols.}}$  is .81, and for fat 0.71. In

experiments in which the object of the research is to determine the type of foodstuff being burned in the body, the respiratory quotient is of special importance. It is used also in the estimation of the basal metabolism by the indirect method since with a respiratory quotient and a known amount of carbon dioxide excreted or of oxygen consumed, the number of calories that are used in the body may be computed by consulting the table of Zuntz and Schumberg.<sup>1</sup>

These investigators found that for a given respiratory quotient one liter or one gram of oxygen represented the combustion of a definite number of calories. Their table also gives in a similar manner the calorific value of carbon dioxide.

Fig. 1 gives the diagram of the Benedict respiration apparatus employed in the infant work at the Massachusetts General Hospital. A detailed descrip-

\*From the Children's Medical Service, Massachusetts General Hospital.

tion of the apparatus may be found in an earlier publication by Benedict and Talbot<sup>2</sup> from which this figure is taken.

A water jacket forms an air-tight seal about the chamber and minimizes the effect of the room temperature on the temperature of the chamber. The air is drawn by a rotary pump from the chamber over a wet and dry bulb psychrometer, (the humidity indicator). The exhaled water is removed by Williams bottles containing sulphuric acid. The dry air then passes through a set of soda lime and Williams bottles which absorb all the carbon dioxide, and before going back into the chamber, the dry air passes through a distilled water bottle to restore a comfortable amount of moisture. During the experiment enough oxygen is admitted from a tank to maintain a slight excess over the amount needed. An expansion or contraction of air is indicated on the spirometer. Thermometers register the temperature of the chamber. The atmospheric pressure is read on a barometer in the room. The figures of oxygen

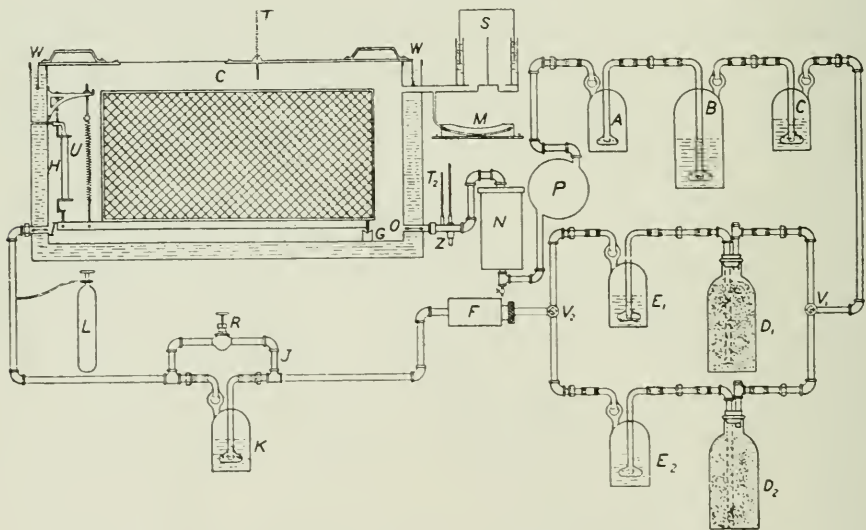


Fig. 1.—Detailed scheme of respiration apparatus. C, chamber; W, W', water jacket; O, outgoing air-pipe; Z, psychrometer; N, Muffler; P, blower; A, acid trap; B and C, Williams water-absorbers; I<sub>1</sub> and I<sub>2</sub>, 2-way valves; D<sub>1</sub> and D<sub>2</sub> carbon-dioxide absorbers; E<sub>1</sub> and E<sub>2</sub>, air-dryers; F, sodium bicarbonate can; J, by-pass; R, valve; K, air moistener; L, oxygen cylinder; I, ingoing air-pipe; S, spirometer; T and T<sub>2</sub>, thermometers; M, manometer; U, spiral spring; H, pneumograph.

read on the oxygen meter\* must be corrected to allow for changes in the volume due to temperature, humidity, and barometric variations in the system. By noting the increase of weight of the set of carbon dioxide absorbers, the weight of carbon dioxide absorbed may be determined.

At frequent intervals we run an alcohol check to determine the accuracy of the apparatus. (For a detailed description of the alcohol check see Publication No. 201, Carnegie Institution of Washington, p. 46.) In brief it is performed in the following manner:

A small alcohol lamp is burned in the chamber, the flame consuming oxygen as the alcohol burns and giving off carbon dioxide thus producing a

\*The oxygen meter measures the oxygen admitted through it from the large tank, but is not shown in the illustration.

condition similar to the combustion mechanism of the body in that oxygen is consumed and carbon dioxide is given off. If the theoretically correct amount of these two gases can be measured in the case of the alcohol combustion, it is safe to assume that the technic and the apparatus are correct for studying the metabolism of the living subject.

When alcohol combines with oxygen, the following reaction takes place— $2C_2H_5OH + 3O_2 \rightarrow 2CO_2 + 3H_2O$ . Since equal volumes of gases at the same temperature and pressure contain equal numbers of molecules (Avogadro's law) it is evident from the above equation that three volumes of oxygen absorbed produce two volumes of carbon dioxide during an alcohol combustion. Hence the respiratory quotient for alcohol is  $\frac{\text{vol. } CO_2}{\text{vol. } O_2} = \frac{2}{3} = .666$ . So if

the measured amount of  $CO_2$  is just  $\frac{2}{3}$  of the volume of oxygen, the alcohol check is successful.

The alcohol check itself is made by lighting the lamp inside the chamber, closing the chamber, inserting the thermometers in the openings, and starting the motor, the air being then set in motion throughout the closed circuit. After the usual corrections have been made, the true volume of oxygen used during the period is ascertained. The respiratory quotient may then be determined by dividing the volume of carbon dioxide by the volume of oxygen.

The most obvious source of error comes from leaks in the tubing or connections. A leak into the system will increase the volume of gas in the system so that it will appear that oxygen had not been consumed, and consequently too little oxygen will be admitted. The figures of used oxygen will then be too low while those of carbon dioxide will be correct, and the result will be too high a respiratory quotient. A leak-out at any point of high pressure also gives an incorrect quotient. To avoid an error of this kind a test for tightness should be run before each experiment. Another possible gross error will result from the use of soda lime bottles no longer able to absorb the carbon dioxide. A check on this is obtained by running the air, after it has passed through the soda lime bottles, through a barium hydrate bottle, a precipitate of barium carbonate forming when the soda lime is inefficient.

We made a study of the factors which could cause variations in the respiratory quotient by burning a small alcohol lamp within the chamber, and found, first, that inaccurate results were obtained if the wet bulb became dry, as shown in Table I.

It is important that the linen on the wet bulb thermometer of the psy-

TABLE I

THE EFFECT ON THE RESPIRATORY QUOTIENT OF A PSYCHROMETER WHICH DOES NOT FUNCTION PROPERLY

TIME	R. Q.	COMMENTS
10:37-11:03	.661	Functioning satisfactorily.
11:03-11:32	.678	Linen moist.
11:32-12:02	.631	Linen drying.
12:02-12:34	.619	Linen dry.

chrometer be of the best grade for handkerchiefs,\* and that the capillary action is good. We have found that a small glass bead sewn to the end of the linen is an advantage in preventing folds.

We have also found that the moisture in the system must be relatively constant. Gross changes in the moisture lead to great errors in the results. The effect of suddenly diminishing the humidity of the air is shown in Table II.

TABLE II

THE EFFECT ON THE RESPIRATORY QUOTIENT OF CHANGES OF MOISTURE IN THE SYSTEM

TIME	H <sub>2</sub> O VAPOR	HUMIDITY	R. Q.	COMMENTS
9:57-10:28	17.0	83%	.650	Constant amount of air passing through moisture bottle.
10:28-10:57	16.1	78%	.667	
10:57-11:28	16.2	78%	.674	
11:28-12:02	12.9	59%	.572	No air passing through moisture bottle. Air dry.
12:02-12:34	13.3	60%	.640	Air still dry. Conditions becoming readjusted.

It should be pointed out that in practical work such sudden changes in humidity should never occur. The humidity control device or by-pass of the moistening bottle, is put on for the purpose of diminishing the humidity that sometimes comes after active exercise such as kicking or crying. It requires considerable skill to regulate this part of the apparatus. We have found it wise to make as little change as is possible in the volume of air passing through the moistening bottle, so as to obviate such sudden changes as we produced artificially.

The height of the spirometer at the beginning and end of the period, when the readings are taken, is also of importance. The most accurate results are obtained when it is low, as shown in Table III.

TABLE III

THE EFFECT OF VARYING THE HEIGHT OF THE SPIROMETER BELL, (PERIODS ½ HOUR), ON THE RESPIRATORY QUOTIENT

R. Q.	COMMENTS
.652	Spirometer bell low at beginning and end of period.
.661	
.663	
.672	
.663 Average	
.695	Spirometer bell high at beginning, low at end.
.673	Spirometer bell low at beginning, high at end.
.644	Spirometer bell high at beginning, low at end.
.679	Spirometer bell high at beginning, low at end.
.610	Spirometer bell low at beginning, high at end.
.622	Spirometer bell high at beginning, low at end.
.654 Average	

\*We found the most satisfactory linen was that which had 1200 threads to a yard in the weft and 1400 threads to the yard in the warp.



Since occasionally it is necessary to end with the spirometer relatively high, we have found it essential that the surrounding room temperature should be kept from varying. Sudden changes in temperature will cause very great errors in the results, due to expansion or contraction of air in the spirometer bell, see Table IV.

TABLE IV  
THE EFFECT ON THE RESPIRATORY QUOTIENT WITH SPIROMETER HIGH AND ROOM TEMPERATURE VARIED

TIME	R. Q.	COMMENTS
10:39-10:58	.660	Room temperature varied from 25.0 to 25.8° C.
10:58-11:31	.600	Room cooled rapidly, temperature dropped to 18.5° C.
11:31-12:03	.446	Continued cooling, temperature dropped to 15.5° C.
12:03-12:37	.624	Room warmed again, temperature rose to 19.8° C.

Such sudden and marked changes in the room temperature ought, of course, never to occur, but they show how change in the temperature will cause incorrect figures of the respiratory quotient. As a matter of fact, the surrounding room temperature should be kept constant and any accidental changes of more than two or three degrees centigrade during a short period should cause results to be looked upon with suspicion.

The results obtained with constant room temperature, constant humidity in the system, and the spirometer low when the readings were taken, are shown in Table V.

TABLE V  
OPTIMUM CONDITIONS, ( $\frac{1}{2}$  HOUR PERIODS)

R. Q.	COMMENTS
.670	Moisture constant, room temperature even, spirometer low.
.658	
.661	
.672	
.665 Average	

#### SUMMARY

The factors essential in obtaining a correct respiratory quotient are first, and most important, an air-tight apparatus and absolutely accurate technic, and, second, all experimental conditions constant. The room temperature should be kept constant and the same as that of the chamber. Sudden variation in the amount of moisture in the system may result in an unreliable respiratory quotient. Variations in these factors are less serious, however, in experiments extending over several hours than in shorter periods. It is of importance to avoid sudden changes in any of the variable factors. If these precautions are taken we feel that very accurate respiratory quotients can be obtained.

This paper does not take into consideration the expulsion of  $\text{CO}_2$  as described by Fries.<sup>3</sup> His work merely emphasizes the fact that respiratory quotients obtained for short periods are more open to error than those obtained from long periods.

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<sup>2</sup>Benedict and Talbot: *Gaseous Exchange of Infants*, Carnegie Institution of Washington, Pub. No. 201, p. 32.  
<sup>3</sup>Fries: *The Respiratory Quotient and Its Uncertainty*, Am. Jour. Physiology, 1921, lv, No. 1.

## A CASE OF TUBERCULOMA OF THE SPINAL CORD\*

BY JAMES J. WARING, M.D., DENVER, COLORADO

THE following is the report of a case of pulmonary tuberculosis complicated by prostatic abscess and a macroscopic intramedullary tuberculoma of the spinal cord.

Patient E. S. P., age twenty-nine, single, by occupation a clerk, presented himself for examination August 4, 1916. The *family history* was important in two particulars only: One half-brother died of tuberculosis of the lungs, and another with tumor of the brain. *Personal History*: Up to the age of 15 years this patient had been very well. Remembers having measles and whooping cough. Did not have scarlet fever, diphtheria, typhoid fever or pneumonia. Has not been subject to tonsillitis or to frequent colds. At about the age of 15 years because of a stubborn cold in the winter he took cod liver oil until the spring. Had a Neisser infection in 1909 for which proper treatment was given. Denies luetic infection and all symptoms suggesting the same.

The first symptoms referable to the lungs were noted in the summer of 1915 when he had some pain in the right side with cough and slight fever. Did not have a pleural effusion but raised some thick yellow sputum, had a few night sweats, no hemorrhages, and considerable lassitude. Tubercle bacilli were found in the sputum July, 1915. He went to the Virginia State Sanatorium during the Winter and stayed there until March, 1916, gaining 24 lbs. in weight, and not being confined to bed at any time. His average weight during the preceding five years was 140 pounds. In July, 1915, at the onset of his lung trouble, his weight 124 pounds. At the time he first consulted me, August 4, 1916, his weight was 135 pounds. The chief complaint at that time was referable to stomach and bowels. He had lost 8 pounds during the previous two weeks, had six to eight stools daily without pain and fever. Temperature normal, pulse 80, urinalysis normal, sputum showed no tubercle bacilli.

Physical examination revealed considerable moisture in the upper lobe on the right side, front and back. A few fine moist râles were heard near the angle of the left scapula behind. His right vocal cord was thick, red and showed definite, though very slight, tuberculous infiltration. Under appropriate treatment he gained to 150 pounds in weight, moisture diminished considerably in his chest; his general condition was most satisfactory.

On November 6 he complained of burning pain in the urethra on voiding and a very frequent desire to empty the bladder. Examination of the urine showed the faintest possible trace of albumin and a considerable number of pus cells. I made a rectal examination and found a large fluctuating nodule about the size of a hazel nut in the left lobe of the prostate. He was referred to a genitourinary specialist with a diagnosis of prostatic abscess, which diagnosis was confirmed, and under treatment the abscess ruptured with a discharge of about 2 oz. of pus through the urethra. On November 20 a pure culture of para colon bacillus was isolated from this discharge. A vaccine was made and administration begun with it immediately thereafter. About this time he complained of weakness. I did not see him again until about December 29 when he told me that he was no better and again spoke of weakness in his legs. The embarrassment of his locomotor apparatus increased very rapidly.

\*Read before the Medical Society of the City and County of Denver.

At this time his subjective symptoms were only increasing weakness of the legs and some inability to control their movements. There was no pain in the back, no girdle sensation, no intercostal neuralgia, and no parasthetic symptoms further than slight numbness of the legs. As long as six months before the onset of the spinal cord symptoms I had noticed his rather curious, studied gait, but considered it merely an individual characteristic. It is possible that his bowel disturbance at his first visit and his peculiar gait at that time were really early signs of pressure in the cord. Early in January this gait was definitely purposeful. His movements in rising from a chair were calculated and there was noticeable adductor spasm during progression and a slight tendency to drag the toes on the right side. The mental condition was perfectly clear. The pupils were equal, regular and reacted to light and accommodation. There was no ptosis, and no squint. No objective or subjective signs referable to the upper extremities or to the upper portion of the body were demonstrable. No fibrillary twitching of the muscles was observed and no evidence of paralysis of the abdominal muscles. There was marked exaggeration of the left knee jerk, slight left patellar clonus, slight left ankle clonus, great spasticity of the adductor muscles on both sides, and marked spasticity of the right lower extremity with exaggeration of all reflexes, positive Babinski and Oppenheim and Gordon reflexes on the left side and a positive Babinski on the right. The cremasteric reflexes and abdominal reflexes were present and active on both sides. An area of diminished sensibility to touch, pain and temperature began about two inches above the umbilicus. From about the level of the umbilicus on the left there was marked diminished sensibility to pain and temperature all the way to the foot. He was unable to distinguish moderate degrees of heat and cold and a sharp pin point was felt as a dull sensation. Ordinary tactile sensation as demonstrated by touching with a piece of cotton was not diminished on the left. On the right there was, from the umbilicus down, slightly diminished sensibility to pain, but not by any means to the extent demonstrated on the left. Sensation remained practically the same from that date to the time of operation with the exception that he showed less dissociation and more marked anesthesia on the left side and the upper level of hypesthesia extended as high as the ensiform cartilage.

The gravity of the motor symptoms progressed with extraordinary rapidity during the last three weeks, the patient losing control of his legs almost entirely. There was slight dragging of the toes on the left side, and very marked dragging on the right side and considerable weakness of the quadriceps extensor group of muscles and even some weakness of the flexors of the thigh. Slight motor weakness on the left side. An effort was made to locate an upper level of hyperesthesia, but no very clear zone could be determined. Muscle sense was unaffected in both lower extremities and there was no loss of the sense of vibration, pall-anesthesia of Oppenheim. Wassermann reaction on the blood, January 1, was negative. Spinal fluid was withdrawn, pressure being slightly increased. Fluid was colorless, not turbid, globulin normal in amount, cell count 4 cells per cubic millimeter, no organisms found. A Wassermann reaction on the spinal fluid was negative, and the curve of the colloidal gold test was normal.

He was seen in consultation by Dr. George Neuhaus about January 15, the symptoms at this time suggesting very strongly syringomyelia. By January 27 he had complete paralysis of his bladder with retention, his bowels moved only with enema. With a view of ruling out tuberculosis of the spine I had several x-ray pictures taken by Dr. S. B. Childs, but there was apparently no disease of the bones.

In spite of the absence of a history suggesting syphilis, the negative blood Wassermann and negative colloidal gold reaction, it seemed wise to give him the benefit of antisyphilitic treatment. Accordingly I gave him cautiously 1/20 of a grain of bichloride of mercury intravenously; in two days gave him 1/10 of a grain, and again in two days 1/5 of a grain. Within a few hours after receiving the third dose he had a terrific attack of diarrhea, bowels, moving fifteen times or more in the next 24 hours, with considerable blood and mucus in the stool. This very intolerance of mercury was the final convincing argument against syphilitic infection and from this time lues as an etiologic factor was discarded.

For good and sufficient reasons it seemed wise to send the patient to Baltimore where he could meet his brother and be operated on at the Johns Hopkins Hospital. He was referred to Dr. Harry Thomas, and in a letter to him dated January 27, 1917, I said:

"Our opinion is that he has myelitis and we are unwilling to say at this time whether it is due to pressure or whether it is an inflammatory condition secondary to his prostatic abscess. If it is due to pressure, the most probable cause would be a tuberculous exudate, pachymeningitis or abscess—at any rate a tuberculous granuloma."

*Operative Notes.*—He was operated on by Dr. George Heuer, whose notes on operation I will now quote:

"Incision was made from the 2nd to the 7th thoracic spine. The lamina of the 3rd to the 7th spines removed, exposing the cord surrounded by relatively little spinal fluid. Over the posterior surface of the cord were numerous small scale-like patches of white material less than half a millimeter in diameter. At the level of the 5th thoracic segment the cord was distinctly hard on palpation and presented increased vascularity at this point. After thoroughly exploring both anterior and posterior surfaces in this region, the cord was incised vertically in the midline, exposing a sharply circumscribed tumor about 2 cm. long, about 4 mm. in width and  $\frac{2}{3}$  mm. in thickness. It was shelled out by blunt dissection without difficulty. The dura was closed tight, and the muscles and skin closed in the usual manner."

Microscopic examination at this time showed the tumor to be tuberculous.

*Subsequent History.*—About the middle of April there had been slight improvement in his legs and he was at times able to perform very slight voluntary movements. His condition, however, was practically the same with the bowel, bladder, and sensory condition very little changed. He died some four and one-half months after operation. Notes on his subsequent history were gathered from reports from his medical attendants in the East.

*General Discussion.*—In the *Annals of Surgery*, February, 1917, lxy, p. 269, Dr. Chas. A. Elsberg reports a case presented before the Philadelphia Academy of Surgery. This man had been operated upon two years previously on account of loss of power in the lower extremities, bladder and rectal disturbances, and severe pain in the lower abdomen. The arches of the 9th, 10th and 11th dorsal vertebrae were resected. A small tuberculous tumor  $1\frac{1}{2} \times 1$  cm. was removed from the substances of the cord itself. Posterior root section was done three months later to relieve the spastic paraplegia. Great improvement followed with recovery of control of the bladder and the ability to walk unsupported.

Veraguth and Brun<sup>2</sup> reported another case in which a conglomerate tubercle was removed from the substances of the cord.

In making a diagnosis the following conditions were considered: Some manifestation of syphilis, some manifestation of tuberculosis, a benign or a malignant tumor of the spinal cord, and a toxic or infectious myelitis secondary to the prostatic abscess.

For the reasons above stated syphilis was excluded. At first I was inclined to consider the condition as inflammatory in nature and secondary to the prostatic abscess. Certainly this latter condition was worthy of consideration as an etiologic factor.

It was immediately apparent in December that there was a spinal lesion of some sort. That it was not an ordinary tuberculous meningitis, a basilar meningitis was equally evident. There was some rigidity of the spinal column, some muscular spasm throughout the lower extremities but weakness without pain. The entire absence of root symptoms such as severe pain and paresthesia before the appearance of marked symptoms referable to the cord indicated clearly pressure, if pressure at all, from within and not from without. I believe it to be a neurological dictum that a painful beginning is most often met with in extramedullary newgrowths. Characteristic of intramedullary pressure were the early motor symptoms, muscular weakness with gradual extension from below upwards and sensory disturbances of the dissociated type. This area of dissociation of sensation was not coincident with the entire area of sensory disturbance but almost so, and with the absence of root symptoms was, to my mind, an invaluable indication of intramedullary disease. The gradual shifting upward of the level, particularly of motor disturbance and partly, though less so, of sensory disturbance, was also suggestive of an intramedullary condition. Extramedullary growths usually enlarge, I believe, in the transverse direction, although a collection of fluid above the growth may give the higher level signs. Intramedullary growths are most common



in the cervical and upper dorsal cord and extramedullary growths may be found in any part of the spinal cord, but are most frequent in the dorsal region. These considerations, naturally, ruled out a spinal complication of Pott's disease. Further, the characteristic complaints of pain in the back or in the area of distribution of one of the nerve roots, the complaint of a girdle sensation were all absent. Also, there was no deformity. Aside from tuberculosis of the arches or the bodies of the vertebrae, tuberculosis may occur in the spinal dura mater or in the substance of the cord and even a localized serous meningitis may complicate tuberculous bone disease.

Elsberg<sup>1</sup> also notes that a chronic edema of the cord over a localized area frequently accompanies tuberculous disease of the vertebrae and dura and may be followed by a toxic softening of the cord at the affected level and by secondary ascending and descending degeneration of the fiber tracts. Sometimes there are even marked cord symptoms without any characteristic macroscopic cord lesion. Elsberg reports one case with a complete paraplegia in which the postmortem examination failed to show any evidence that the cord had been compressed or diseased. Tuberculous disease of the arachnoid and inner surfaces of the dura is rare, while tuberculous abscesses and masses of granulation tissue on the outer surfaces of the dura are frequently met. There is often a thick mass of granulation tissue adherent to the outer surfaces of the dura. Not so rarely the dura itself is the seat of a localized tuberculous pachymeningitis which results in marked thickness so that the cord is markedly compressed with symptoms much like those of spinal tumor. Spinal cord symptoms in tuberculosis may be due further to compression of the cord through the dura by dislocated bone, encapsulated masses of cheesy material or by newly formed bone which causes a marked narrowing of the spinal canal. Parenthetically it may be noted that there may be marked deformity of the spine from Pott's disease without any compression of the cord.

Finally, the existence of tuberculosis of the lungs, the absence of symptoms and physical signs and x-ray evidence of Pott's disease, the preponderating indications of an intramedullary condition, all prompted a positive diagnosis of tuberculoma of the spinal cord with the possible reservation of a benign tumor of the spinal cord.

I am indebted to Dr. George Neuhaus for his skilled counsel in consultation and analysis of this case.

#### REFERENCES

<sup>1</sup>Elsberg: Diseases of the Spinal Cord and its Membranes.

<sup>2</sup>Veraguth and Brun: (a) *Cor. Bl. f. schweiz Aerzte*, 1919, xl, 1697. (b) *Ibid.*, 1916, xlv, 385, 424.

## ISOLATION OF TETANUS BACILLI FROM THE CEREBROSPINAL FLUID. REPORT OF A CASE\*

BY G. R. LACY, M.D., AND CECILIA MURDOCK, B.S., PITTSBURGH, PA.

IN TETANUS infections of the human being, it is generally conceded that the spores of the bacillus are not transported to other parts of the body, but that they remain localized at the point of inoculation, and that the symptoms produced are due to an absorption of the toxin developed at the primary focus. Hochsinger<sup>1</sup> and Creite<sup>2</sup> reported exceptions to this rule, however, when they succeeded in isolating tetanus bacilli from heart blood and spleen of infected human beings. It was also demonstrated experimentally, by Vailard,<sup>3</sup> Tarozzi,<sup>4</sup> Canfora,<sup>5</sup> and others that when animals were inoculated subcutaneously with detoxicated tetanus spores, the spores were readily phagocyted and carried to various parts of the body, where they could lie dormant for several weeks. It was shown further, that all that was necessary for the development of tetanus in these cases was the production of a simple wound, the fracture of a bone, or the introduction of some form of pyogenic bacteria. The explanation of this factor probably is that the tetanus spores can exist without multiplication in living active tissue where the supply of oxygen is normal, but that as soon as the tissue becomes devitalized, as a result of injury, and is no longer freely supplied with oxygen, it becomes a favorable field for the growth and development of the anaerobic tetanus bacillus. It seems possible, therefore, that man may be inoculated with low grade tetanus spores which are rapidly phagocyted and carried to distant parts of the body where they may lie dormant until some factor arises which will permit them to develop. The case which we are reporting may be explained in this manner.

### CASE REPORT

H. C., colored, laborer, age twenty-four years, was admitted to the Allegheny General Hospital July 17, 1920, complaining of enlargement of the scrotum.

Patient's mother and grandmother had died of tuberculosis, his family history otherwise unimportant.

Patient had whooping cough when 3 years of age and had pneumonia during the summer of 1919, being confined to bed for 2 months. He had had gonorrhea twice within the past two years, but denied ever having had lues. His Wassermann test was negative. About six weeks previous to entrance into hospital, painless swelling of the testicles was noted. This swelling persisted without other symptoms until about four weeks before entrance, when erosion of the right side of the scrotum developed. Beginning as a small sore, it soon involved the entire right side of the scrotum and a small area on the left. When he was admitted to the hospital, real pain was absent, but he complained of a stinging sensation at the site of erosion.

Physical examination showed heart and vessels normal, rhythm regular, a few crackling râles in the lungs, abdomen and extremities normal. There was an enlargement of scrotum and testicles. The skin had practically all sloughed from the right side of the scrotum

\*From Wm. H. Singer Memorial Research Laboratory, Pittsburgh, Pa.

and from a small area about the size of a five cent piece on the left side of the scrotum, leaving a raw bleeding surface.

The patient was treated with local applications and given one injection of salvarsan during the first three weeks in the hospital. No improvement was noted. Many acid fast bacilli were found in a twenty-four hour specimen of urine, July 19.

August 9, under general anesthetic, the right testicle was removed. The pathologic report was tuberculous orchitis and epididymitis. He presented the usual postoperative development until August 21, twelve days after operation, when he began to complain of severe pains in the back and back of the neck. At this time he was given 10,000 units of tetanus antitoxin subcutaneously. August 22, patient showed well-developed clinical tetanus and was given 2 intravenous and one intramuscular injection of 10,000 units of antitoxin, making a total of 30,000 units in 24 hours. On the 23rd he was given 2 injections of antitoxin, 10,000 units each, and on the 24th a similar amount.

Patient continued in great distress and his condition becoming critical, lumbar puncture was done under chloroform anesthesia on the 25th and 10,000 units of antitoxin were administered intraspinaly. Antitoxin (10,000 units intramuscularly) on the two subsequent days failed to give immediate relief of symptoms. At this time, August 27, patient developed hypersensitiveness to the serum and such treatment was discontinued. During the course of antitoxin treatment, a period of seven days, the patient had received 110,000 units. Sedatives were administered to alleviate the severe pain throughout the period of infection. Improvement was gradual and uninterrupted from the cessation of serum therapy until the time of complete recovery and patient was discharged from the hospital September 15, 1920.

*Bacteriologic Findings.*—A sample of cerebral spinal fluid was received at the Laboratory, August 25, 1920, with a request for culture for the tetanus bacillus. The fluid was practically clear and smears from the sediment of a centrifuged specimen were negative for cells and for bacteria. A guinea pig was inoculated with  $\frac{1}{2}$  c.c. of the centrifuged fluid and failed to show any signs of infection during the period of two months' observation.

*Cultures.*—Deep dextrose agar stick, litmus milk, and cooked meat medium were each inoculated with the spinal fluid and incubated at 37° C. In 24 hours there was no apparent growth, but in 48 hours there were gas bubbles appearing in the dextrose agar stick. Preparations of this showed motile gram positive bacilli. In 72 hours these bacilli contained the characteristic racket-shaped terminal spores. At this time the same type of bacilli was found in the meat medium. Anaerobic plates showed pure cultures of tetanus bacilli.

On August 31, material was obtained from the operative scrotal wound and an attempt was made to isolate the tetanus bacillus. The cultures remained negative for tetanus bacilli, but contained staphylococcus albus, *B. coli communis*, and *B. Welchii*. Taking for granted that the operative scrotal wound contained the tetanus bacillus at some time during the infection, failure to grow it may have occurred from any one of three causes: first, it is possible to have taken the swab from the wound and missed entirely the area containing the tetanus bacilli; second, the bacilli may have been in the deeper tissues and been missed by the swab from the surface; third, it seems more probable that the patient had been able under the antitetanic treatment to destroy the bacilli since the same methods were used as in culturing the spinal fluid.

Feeling that there might be a question of the identity of the organism isolated from the cerebral spinal fluid, we did the following animal experiments:

**GUINEA PIG 1.**—August 28, 1920, a 200 gram G. P. was injected in the muscles of the right hind leg with  $\frac{1}{2}$  c.c. of saline suspension of the organisms from the original dextrose agar culture. The pig was found dead in the cage 20 hours after inoculation. Autopsy showed only an extensive hemorrhagic area at the site of inoculation. Cultures from this area contained pure tetanus bacilli.

**GUINEA PIG 2.**—August 30, 1920, a 250 gram G. P. was injected in the muscles of the right hind leg at 4 P.M. with  $\frac{1}{4}$  c.c. saline suspension of organisms from the dextrose agar stick. At 9 A.M., August 31 there was a complete spastic paralysis of the inoculated leg, and some impairment of motion in the other hind leg. The animal was highly nervous,

frequently developing typical tetanic convulsions when frightened. At 10:30 A.M. blood was taken from the heart with sterile needle and syringe for culture and for injection into another animal. Death occurred during the bleeding. Autopsy showed only a slight amount of hemorrhage at the point of inoculation. Grossly the abdominal organs appeared negative. Cultures from the heart's blood remained negative. Results of the inoculation with the heart's blood will be given under the next experiment.

GUINEA PIG 3.—August 31, 1920, at 10:30 A.M., a 250 gram G. P. was injected in the muscles of the right hind leg with a little less than 2 c.c. of heart's blood from G. P. 2. At 4:30 P.M. there was not a definite paralysis but the pig favored the injected leg in walking. At 9 A.M., Sept. 1, there was a complete rigid paralysis of the inoculated leg, but the other legs seemed uninvolved. Paralysis gradually extended throughout the day so that at 3 P.M. there was impairment of motion in the other hind leg also. At this time blood was taken from its heart for culture and for injection into another animal. At 5 P.M. there was complete paralysis of both hind legs but of less spastic type in the left than in the right. At 9 A.M., September 2, the pig was found dead in the cage. At autopsy there was a marked rigidity of the right hind leg but very little rigidity of the left and of the front legs. Around the point of inoculation there was a slight hemorrhagic condition which might have been due to the blood injected. The liver showed an acute hemorrhagic hepatitis. Kidneys showed acute tubular and glomerular nephritis, the whole lining epithelium being extensively involved. Culture from the heart's blood remained negative. The other animal injected with this pig's blood did not show any signs of tetanus during 2 months' observation.

GUINEA PIG 4.—August 30, 1920, a 200 gram G. P. was injected subcutaneously with  $\frac{1}{2}$  c.c. of Mulford's antitetanic serum. No ill effects observed. On August 31 the same pig was inoculated in the muscles of the right hind leg with  $\frac{1}{4}$  c.c. saline suspension from the original dextrose agar. There were no signs of tetanus developed during the period of two months' observation.

GUINEA PIG 5.—August 31, 1920, a 175 gram G. P. was injected in the muscles of the right hind leg with  $\frac{1}{2}$  c.c. of saline suspension of the original dextrose agar culture which had been boiled for 1 minute just prior to injection. No signs of infection developed during the period of 2 months' observation.

GUINEA PIG 6.—August 31, 1920, at 4:00 P.M., a 250 gram G. P. was injected subcutaneously with 1 c.c. of Mulford's antitetanic serum representing approximately 300 units. In ten minutes,  $\frac{1}{4}$  c.c. of the same culture as used in the other pigs was injected intramuscularly into the right hind leg. At 9 A.M., August 31, there was slight impairment of motion in the inoculated leg. The impairment of motion gradually advanced until 1 P.M. when the animal was given another 1 c.c. of antitoxin subcutaneously. The leg was then in a state of complete rigid paralysis. This condition lasted throughout the rest of the day and appeared to be even worse on the following day. Sept. 2, the leg was still in extreme spastic paralysis but the pig was spry and ate his food readily. Contraction of the muscles of the leg continued until Sept. 7, when the rigidity began gradually to disappear. From Sept. 10 until Sept. 17 the animal was able to walk slowly on the affected leg. On Sept. 17 there was a recurrence of symptoms at which time the left hind leg was also somewhat involved. Sept. 18, complete loss of control of both hind legs, extreme nervousness and opisthotonus developed. 500 units of antitoxin were given subcutaneously. Pig died during the night of Sept. 18. At autopsy the abdominal organs appeared to be normal and all cultures were negative for tetanus bacilli.

#### DISCUSSION

Guinea pigs Nos. 1 and 2 reacted exactly as one would expect from non-protected animals. The 2 c.c. of heart's blood of pig No. 2 contained enough toxin to cause the death of pig No. 3, but it is evident that the circulating blood did not contain tetanus bacilli, since the cultures from both pigs remained



negative and the blood from pig No. 3 failed to produce any symptoms when injected into another animal. Pig No. 6 showed the delayed production of symptoms when antitoxin was given concurrent with the culture, and the gradual improvement followed by death 19 days after inoculation, due probably to insufficient amount of antitoxin. Pig No. 4 showed the results of immunization previous to inoculation, and pig No. 5 showed that boiling was detrimental to the toxin and if the spores were not killed also, that the animal was able to render them incapable of producing the disease.

The cultural findings and the animal inoculations proved conclusively that the bacillus isolated was the tetanus bacillus.

The use of surgical precautions in making the spinal puncture, the recovery of the tetanus bacillus in pure culture, and in considerable numbers, as shown by the number of colonies in the agar stick, almost excludes the possibility of a contamination of the spinal fluid at the time of collection.

The way in which the bacilli reached the spinal fluid is not entirely clear since it is not known whether the patient became infected with the tetanus bacillus shortly prior to his operation, during it, or subsequent to it. There was no history of injury which would lead to direct infection of the meninges. There was, however, ample opportunity for inoculation prior to operation since there had been a large open scrotal wound for many days. The close proximity to the anal outlet would make it very easy for inoculation to take place from the feces. It would seem that this is the most likely source of infection as indicated by the presence of *B. coli* and *B. Welchii* in the wound. For the bacilli to have reached the spinal canal, there must have been either a blood stream infection with the bacilli living and multiplying in the blood, or phagocytosis and transportation of the spores to the spinal canal where they were deposited and remained dormant until conditions developed favorable for their growth and reproduction.

Whatever the explanation for its occurrence, we feel that on account of the rarity of the case, it should be reported since all teachings have been that the tetanus bacillus does not occur in the cerebrospinal fluid.

We desire to express our thanks to Dr. James C. Burt for the privilege of reporting this case.

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- <sup>4</sup>Tarozzi: *Centralbl. f. Bakt.*, 1 abt., Jena, 1905-6, xl, 305.
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## SOME DIFFERENCES IN RESPONSE TO ATROPINE IN WHITE AND COLORED RACES\*

BY H. A. PASKIND, B.S., M.D., CHICAGO, ILL.

IT is known to pharmacologists that atropine varies in its effect in different animals. For example, rabbits and guinea pigs are relatively insusceptible to its action. In a study of the action of small doses of atropine in man McGuigan† found that small doses of atropine always cause slowing of the pulse, and he also noticed indications of differences in the susceptibility in the white and colored patients as evidenced by the action on the vagus center. The present investigation is concerned with that difference. In this study I have compared the action of small doses of atropine on twenty white and twenty negro patients. These patients were about the same weight and were selected from the genitourinary wards of the hospital. Their indisposition consisted mainly in secondary syphilis, chancre, or gonorrhea. Their temperature was normal and for the testing of this drug, they may be considered as normal. I do not think there was anything in their condition that would in the least modify the action of the drug which conforms to results recorded for normal conditions.

### METHOD

The method used consisted in the hypodermic injection of atropine sulphate, (.2 per cent) 1 c.c. of this is equivalent to 0.002 milligram or 1/30 grain. Injections were all made under similar conditions. The patient sat in a chair while the observations were made. No bedridden or acute cases were used. The heart-rate was observed for some time before the injection and when found to be uniform, injection was made and records kept every five minutes for an hour. The twenty men in both groups were approximately the same weight and age. The colored men varied from 135 pounds to 175 pounds with an average of 158 pounds. The age varied from twenty-two years to forty years with an average of twenty-eight years.

The weight of the white men varied from 135 pounds to 172 pounds with an average of 152 pounds. The ages of the whites were from 20 years to 54 years with an average of 32 years.

The following three cases of each group are selected as showing the typical effect of small doses of atropine on the heart rate.

In these two typical groups the initial heart rate is practically the same but in the colored patients atropine causes no initial slowing of the heart while in the whites, initial slowing is marked. The acceleration is practically the same in each case. Slowing of the pulse rate is appreciable in the white men in about five minutes and reaches the greatest slowing in ten to fifteen minutes. Acceleration begins with the dosage used in about thirty minutes, which is about the onset of

\*From the Laboratory of Pharmacology, University of Illinois College of Medicine and Cook County Hospital, Chicago.

†Jour. Am. Med. Assn., 1921, lxxvi, 1338.

TABLE I  
TYPICAL CASES, COLORED MALES

	HEART RATE PER MINUTE		
	I	II	III
Normal rate per minute	64	84	76
Atropine sulphate gr. 1/60 injected hypodermically.			
After 5 minutes	64	84	76
10 "	64	88	76
15 "	64	96	76
20 "	64	96	76
25 "	72	96	84
30 "	84	96	88
35 "	88	106	88
40 "	96	96	88
45 "	96	100	92
50 "	96	100	88
55 "	100	108	92
60 "	100	108	88
Greatest slowing of heart rate	0	0	0
Greatest increase in heart rate	36	22	16

TABLE II  
TYPICAL CASES, WHITE MALES

	HEART RATE PER MINUTE		
	I	II	III
Normal rate per minute	60	80	72
Atropine sulphate gr. 1/60 injected hypodermically.			
After 5 minutes	56	76	68
10 "	48	64	60
15 "	48	76	60
20 "	60	84	60
25 "	60	96	72
30 "	68	92	84
35 "	72	104	92
40 "	80	96	96
45 "	76	104	100
50 "	80	100	100
55 "	84	96	100
60 "	84	100	104
Greatest slowing in heart rate	12	16	12
Greatest increase in heart rate	24	24	32

acceleration in the negroes. In white patients where 1/30 grain of atropine is administered, the slowing is very transient and acceleration quickly develops. In negroes 1/120 grain is inactive, on the other hand 1/30 grain may elicit a slow pulse where 1/60 grain fails; for these reasons it is apparent that the negro is less susceptible than the white man. This lesser susceptibility applies to the central effect, or the action on the vagus center only. The peripheral action seems to be the same in both classes of patients.

In a few cases of the negroes, there was some initial slowing, but this was slight while in the white cases practically all showed a depression and those that did not, apparently were so susceptible that acceleration occurred almost immediately. The depression in all cases is marked. Tables III and IV show this clearly:

TABLE III

Colored	R. B.	E. P.	W. M.	J. R.	I. S.	S. B.	A. S.	E. W.	C. W.	T. J.	D.	J. S.	O. R.	L. C.	C. W.	R. P.	A. R.	R. B.	R. C.	A. E.
Normal rate	72	80	84	92	68	84	96	81	98	60	82	64	72	76	64	72	84	68	81	76
Atropine grain	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	60	60	60	60	60	60	60	60	30	30	30	60	60	60	60	60	60	60	60	60
After 5 min.	68	80	81	92	68	84	96	81	96	60	86	64	72	76	64	72	84	68	81	76
" 10 "	72	84	81	88	68	88	92	80	104	60	84	64	76	76	64	72	92	68	92	76
" 15 "	72	96	96	88	72	96	92	81	91	60	82	64	72	81	64	80	108	68	104	76
" 20 "	68	100	88	88	72	96	96	80	116	60	78	68	72	88	64	80	120	68	108	76
" 25 "	80	96	92	88	80	96	91	96	118	72	80	61	80	92	72	80	120	68	120	84
" 30 "	84	120	88	88	80	96	98	96	114	66	$\frac{1}{30}$ gr.	68	80	100	84	88	112	72	120	88
" 35 "	76	120	92	92	80	106	100	96	112	$\frac{1}{30}$ gr.	100	76	96	104	88	88	120	72	120	88
" 40 "	88	112	96	88	80	96	100	92	116	92	104	76	100	104	96	92	120	76	120	88
" 45 "	88	112	104	88	80	100	100	96	152	100	104	76	100	100	96	96	120	76	120	92
" 50 "	88	112	104	96	80	100	100	100	124	104	104	88	104	100	96	100	112	80	120	88
" 55 "	88	120	100	88	84	108	100	104	118	108	104	84	104	100	100	100	112	80	120	92
" 60 "	92	112	100	88	80	108	108	100	120	108	106	88	104	100	100	100	112	80	108	88
Greatest depression	4	0	0	4	0	0	4	4	2	0	4	0	0	0	0	0	0	0	0	0
Greatest acceleration	20	40	20	4	16	22	12	16	54	48	24	24	32	24	36	28	36	12	36	0

Average depression 12  
Average acceleration 25



TABLE IV

White	P. M.	J. A.	B. W.	L. B.	J. R.	L. H.	J. D.	E. C.	M. M.	J. M.	R. H.	J. H.	J. B.	H. B.	J. R.	C. C.	H. K.	P. T.	A. K.	J. W.
Pulse	80	88	80	80	96	88	72	80	84	88	84	88	84	60	96	88	80	86	72	88
Atropine Sulphate Grains 1/60																				
After 5 min.	68	96	72	68	96	84	68	76	68	80	76	88	72	56	112	76	72	80	68	88
" 10 "	84	96	88	68	92	92	60	64	68	92	80	80	72	48	112	96	68	72	60	80
" 15 "	88	96	88	78	96	76	64	76	80	100	80	72	80	48	130	100	68	76	60	84
" 20 "	92	92	88	78	108	96	80	84	76	100	88	76	92	60	134	112	76	80	60	84
" 25 "	96	92	92	84	108	122	80	96	72	100	100	80	100	60	144	112	80	88	72	84
" 30 "	100	88	100	84	120	108	84	92	80	108	100	84	108	68	144	112	88	88	84	96
" 35 "	100	100	100	92	120	120	84	104	88	104	108	88	120	72	154	100	92	88	92	96
" 40 "	112	100	100	96	120	112	92	96	96	108	104	88	120	80	154	108	100	92	96	96
" 45 "	120	112	100	104	116	120	88	104	96	116	112	88	108	76	150	120	96	96	100	96
" 50 "	120	120	100	112	112	124	96	100	96	104	120	88	112	80	150	120	96	96	100	96
" 55 "	120	132	120	112	112	130	96	96	96	108	108	92	108	84	152	112	100	88	100	92
" 60 "	120	135	120	104	120	128	96	100	96	100	104	96	120	84	152	112	100	88	104	96
Greatest depression	12	0	8	12	4	12	12	16	16	8	8	16	12	12	0	12	12	8	12	8
Greatest acceleration	40	47	40	24	24	42	24	20	12	28	36	8	36	24	56	32	20	16	32	8

Average slowing 10  
Average acceleration 28

## RÉSUMÉ

In the colored patients the average normal heart rate was 78; while atropine produced an average of one beat per minute.

In the white group the normal heart rate was 87; and in these cases atropine reduced this rate to 77 or an average slowing of 10 beats per minute.

From the results of our study, therefore, we conclude that the negro is less susceptible than the white man, to the central action of atropine.

# LABORATORY METHODS

## A PRELIMINARY REPORT ON A METHOD OF DETERMINING THE NUMBER OF COMPLEMENT BINDING UNITS IN SERA GIVING POSITIVE WASSERMANN REACTIONS\*

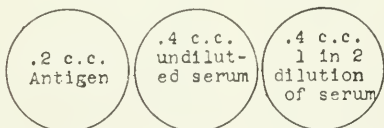
By ARCHIBALD McNEIL, M.D., NEW YORK, N. Y.

FINDING that a large number of sera that give positive Wassermann reactions, contain many more complement binding units than are necessary to give a ++++ reaction, the following titration method was devised to enable the serologist to ascertain the number of complement binding units present in any given serum.

In the classical Wassermann test the complete binding of two units of complement by 0.1 e.e. of serum is considered a ++++ reaction. So taking 0.1 e.e. of serum as a starting point, a series of dilutions is prepared, the difference between each succeeding dilution being exactly one plus.

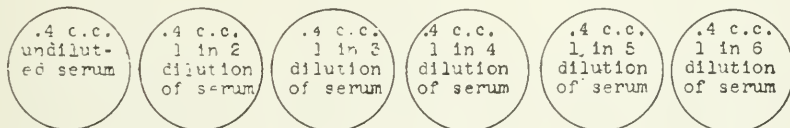
### SET UP OF TITRATION

#### Antigen and serum controls



Complement, 1.0 c.c. 1.0 c.c. 1.0 c.c.  
Saline to bring total volume of each tube to 3.0 c.c.

Serum to be tested,



Antigen, 0.1 c.c. 0.1 c.c. 0.1 c.c. 0.1 c.c. 0.1 c.c. 0.1 c.c.  
Complement, 1.0 c.c. 1.0 c.c. 1.0 c.c. 1.0 c.c. 1.0 c.c. 1.0 c.c.  
Saline to bring total volume of each tube to 3.0 c.c.

In titrating an unknown serum it is advisable to make a preliminary titration using 1 in 10 and 1 in 100 dilutions, employing the same technique as in an amboceptor titration and using 0.1 e.e. of antigen 1.0 e.e. of complement as standardized for the regular test, together with the proper antigen and serum controls. A rough estimate of the total number of complement binding

\*Received for publication, August 20, 1921.

units present may be obtained by this method, saving much time and trouble in preparing unnecessary dilutions.

Shake and place in an ice box for four hours to bind complement. At the end of four hours remove from the ice box and add 2.0 c.c. of sensitized sheep's cells and place in water-bath at 37° C. and allow to remain for fifteen minutes after antigen and serum controls show complete hemolysis. Place in ice box and make reading at the end of twelve hours. The reading is made on the tube containing the least amount of serum that shows complete inhibition of hemolysis. Thus if the tube containing .4 c.c. of a 1 in 5 dilution of

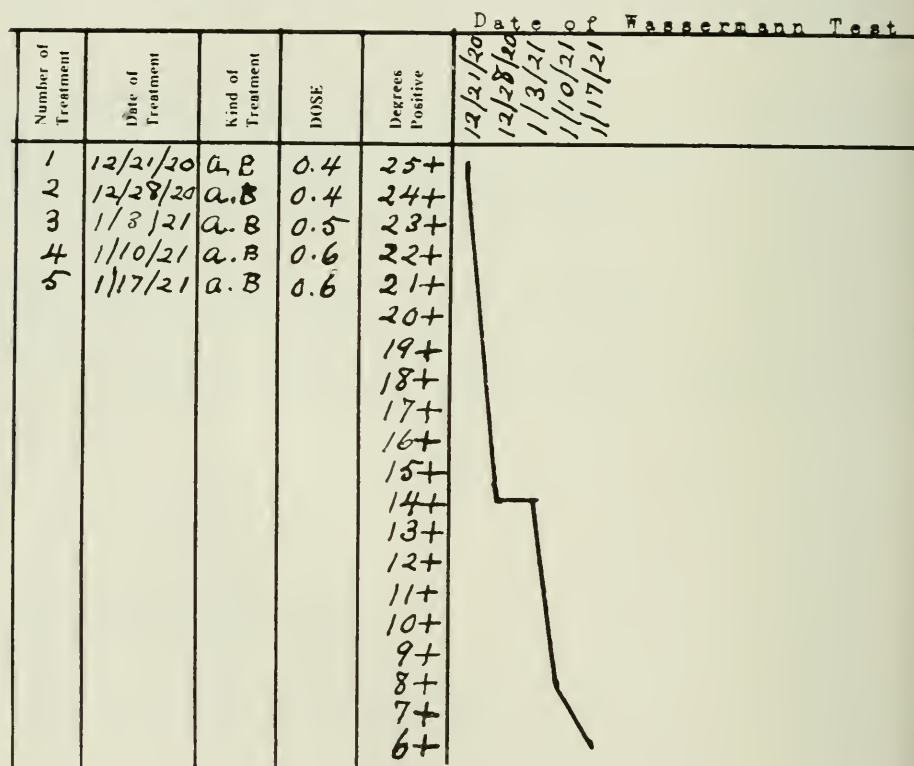


Chart I.

serum shows complete inhibition of hemolysis and the tube containing .4 c.c. of a 1 in 6 dilution shows slight hemolysis the reading will be 5+.

At first an attempt was made to make readings fifteen minutes after the controls showed complete hemolysis at 37° C. in the water-bath, by centrifuging the tubes. But it was found that a considerable variation occurred in the readings of a series of titrations on the same serum with different hemolytic systems; while if the readings were made after a period of twelve hours in the ice box, they were practically the same in all the titrations.

#### REAGENTS

The most satisfactory results have thus far been obtained by using such a dilution of amboceptor, that 1.0 c.c. will contain five Bordet units.



Complement in a 1 to 30 dilution is titrated against 2.0 c.c. of sensitized sheep cells and the reading made after the titration has remained in the water-bath at 37° C. for one half hour, and the dilution of complement is so adjusted that 1.0 c.c. will contain two units of complement. Antigen is used in the same dilution and amount as in the routine Wassermann test, and of course it is necessary to use the same antigen in a constant dilution throughout any given series of titrations.

Chart I is used to record the results of titrations for the benefit of the clinician.

**HISTORY AND TREATMENT OF CASE SHOWN ON CHART 1.**—Male, 37 years old. Married. Patient while shaving with a borrowed razor, one and one-half years ago, cut his left cheek and about two weeks later a chancre developed on the site of the wound; this was followed in due time by general secondary manifestations. (The owner of the razor was suffering from an active and untreated syphilis.)

*Physical Examination.*—Syphilitic Manifestations as above described. Patient well nourished; heart, lungs, kidneys and eyes normal. No symptoms referable to cord. Wasserman 25+.

*Treatment.*—On December 21 and 28, 1920, and January 3, 10, and 17, 1921, arsenobenzol was administered and on December 24 and 31, 1920, and January 6, 1921, injections of salicylate of mercury were given. On December 31, 1920, and January 3, 1921, the Wassermann was 14+, on January 10 it was 8+ and 6+ on January 17. On December 31, 1920, all visible manifestations of syphilis had disappeared and the patient had gained 16 pounds.

Unfortunately there is no further record of this case as business has obliged the patient to move to a distant part of the country where he has been advised to continue the above treatment.

(Signed) J. R. Hayden.

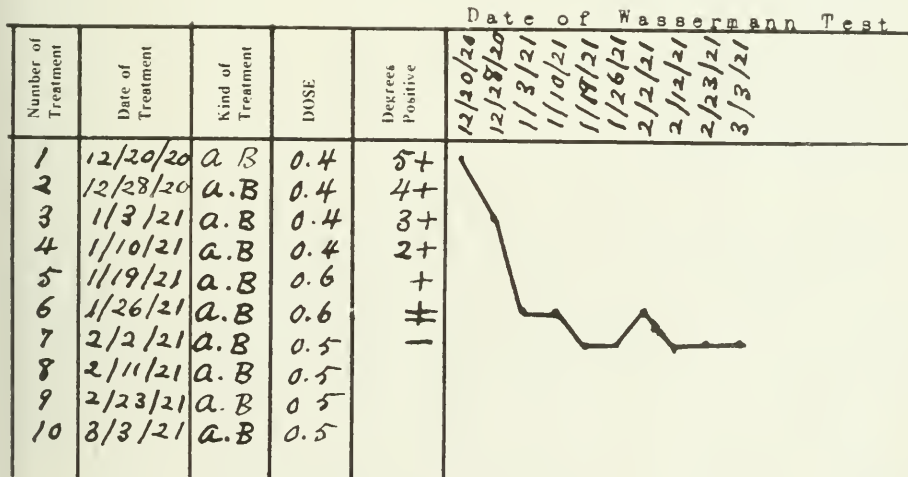


Chart II.

**HISTORY AND TREATMENT OF CASE SHOWN ON CHART NO. 2.**—Male thirty years old, single. Penile chancre in October, 1911.

*Physical Examination.*—Patient well nourished. Reflexes, eyes, heart, and lungs normal. On December 15, 1911, the Wassermann was positive and on the 18th, general secondary manifestations appeared.

*Treatment.*—The regular inunction treatment with 50 per cent mercurial ointment

was ordered and on December 30, the secondary manifestations had practically disappeared. Beginning January 7, 1912, potassium iodide was administered in conjunction with the inunctions. On February 27 and November 18, 1913, Wassermann tests were made with negative results. By February 8, 1914, the patient had received 209 rubbings with mercurial ointment. On May 20, 1914, the Wassermann was weakly positive and on May 25, June 23, 29, and July 7, and 20 neosalvarsan was administered. Wassermanns taken on June 9 and September 21 were both negative. By February 21, 1916, the patient had received 264 rubbings in conjunction with the administration of iodide of potassium. At this time no manifestations of the disease were present and on May 19, August 3, and December 28 the Wassermann was negative. For about five years no antisyphilitic treatment was given and negative Wassermanns were obtained June 29, 1917, and November 29, 1919.

On December 7, 1920 the Wassermann was 5+ and on December 20 and 28, 1920 and on January 3, 10, 19, and 26 and on February 2, 12, 23 and March 3, 1921, arsenobenzol was administered in conjunction with injections of salicylate of mercury on December 23 and 30 1920 and January 7, 14, 22 and 31 and February 7, 15 and 28. On December 29, 1920 the Wassermann was 3+ and on January 4 and 11, 1921, plus-minus, and on January 19 the report was "delayed negative." On February 2 the Wassermann was a plus-minus and on February 23 a delayed negative, and on February 14, 23, and March 3, negative. No further medication has been given and the patient is in fine mental and physical condition at the present time.

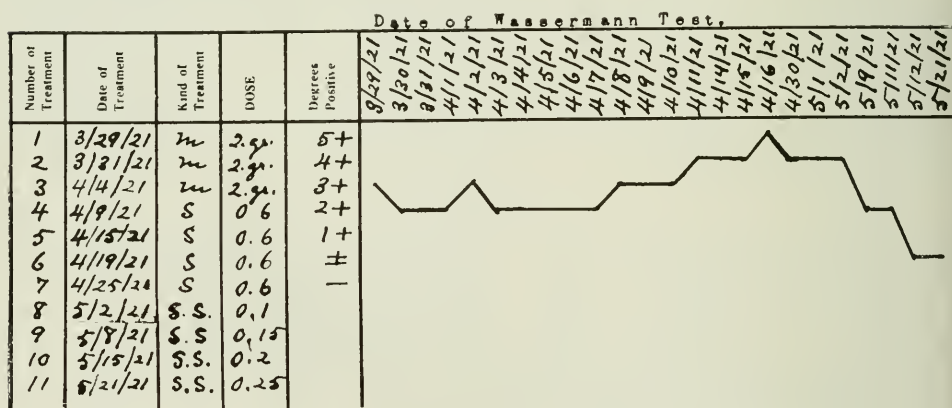


Chart III.

HISTORY AND TREATMENT OF CASE SHOWN ON CHART No. 3. Sex, male. Age thirty-six. Chancre March, 1918. Two weeks after the appearance of the chancre the Wassermann was positive, but there were no secondary lesions. One injection of salvarsan was given at this time. In July, 1918, the Wassermann was positive and injections of salvarsan were given on July 9, 19, and 26 and on August 2 and 26. The Wassermann remained positive after this course of treatment which was accompanied by ten injections of mercury.

During his next vacation in 1919 he received injections of salvarsan on July 8, 18, and 23, and August 6, 18, 29, and November 8. The Wassermann remained positive throughout, with the exception of one taken on August 28, which was reported "negative." During 1919 following a violent reaction to a salvarsan injection he developed a tuberculo-squamous syphilide on the back of the hand and wrist, on the forearm, and on the sacrum. These lesions which have persisted to the present time, are all small and discreet, but distinctly squamous syphilitic infiltration.

In 1920 he received salvarsan on July 20, 22, and 24 and on August 3, 10, 16, and 25. With these treatments he received six injections of mercury. It is believed that all the injections of salvarsan were 0.5 or 0.6 grams.

In March, 1921, physical examination failed to reveal any abnormalities of the reflexes, or suggestion of any other lesion of the nervous system, beyond a blood pressure of 145 systolic and a slight murmur over the aortic region suggesting a possible sclerosis there.

On March 29 and 31 and April 4, intramuscular injections of 2.0 grains of bichloride of mercury in oil were given and on April 9, 15, 19, and 25 arsphenamine (Metz) was given in 0.6 gram doses.

During the administration of mercury the external lesions were uninfluenced and the Wassermann remained substantially stationary at from two- to three-plus. During the administration of salvarsan the Wassermann rose to five-plus and the skin lesions were uninfluenced.

On May 2, 1921, the administration of silver salvarsan was begun. The first dose was 0.1 gram and a week later 0.15 gram was given and so increasing until 0.25 gram was given in the fourth dose on May 21. During this period the Wassermann fell to a plus-minus reaction and the skin lesions were markedly improved, far better the patient said than at any time since their first appearance. The patient was obliged to leave town at this time and has not been under observation since. This influence of silver salvarsan should not be regarded as an essential quality of the drug. It might presumably have been that the treponemata in this case were susceptible to silver salvarsan and not to some other drug.

From March 29 to April 11, 1921, blood specimens were taken for the Wassermann test daily and from that time until May 21, they were taken at frequent intervals.

(Signed) Edward L. Keyes, Jr.

In cases where there are a large number of complement binding units present in the blood when treatment is begun, the regular Wassermann test may show continuously ++++ reactions for a period of weeks or even months, whereas the titration method may show a continuous decrease in the number of complement binding units as the treatment progresses and the patient does not become discouraged and either discontinue treatment altogether or drift from one physician to another as so often happens.

In a more detailed report on the titration method of performing the Wassermann test, a special study will be made of the so-called "Wassermann fast" cases, with regard to the possibility that they are in the majority of instances, simply cases in which the treponemata are immune to the drug or drugs that have been administered for a considerable period of time.

Experimental work is at present being carried on to see whether it is possible by the titration method to determine when the treponemata in any given case have become immune to a certain drug or drugs and when a change in treatment is indicated.

Another interesting study that is being carried out by the titration method, is to determine the number of hours in which a single dose of the various drugs that are used in the treatment of lues will show their maximum effect on the Wassermann reaction.

I wish to thank Doctors James R. Hayden and Edward L. Keyes, Jr., who have kindly supplied the clinical data from which the charts were prepared.

## A RAPID METHOD OF PREPARING TISSUES FOR MICROSCOPIC EXAMINATION\*

By HERMAN D. MELTON, RICHMOND, VA.

IT IS often desirable to prepare tissues for microscopic examination with as little delay as possible. Of course, the frozen section method is the most rapid available, enabling a diagnosis to be made during the operation. This method, however, often leaves much to be desired in the clearness of the sections; so serious a disadvantage in certain instances as to render accurate diagnosis difficult or impossible. For several years, I have been engaged in the attempt to devise a method which will approach the frozen-section method in speed, but, by means of which, more satisfactory sections could be obtained. As a result of these efforts, the following procedure has been devised.

If the tissue to be examined is fresh, it is cut into small blocks, about 5 m.m. One or more of the blocks is covered with 4 per cent formaldehyde contained in a test tube, and the solution boiled over the free flame for 3 minutes. The formaldehyde is poured off and the block covered with tap water. The water is boiled over the free flame for 1 minute; poured off; fresh water added; and the boiling repeated twice. In all, three changes of water are used with the 1 minute interval of boiling in each case. If the tissue has already been fixed in formaldehyde, the preliminary boiling in formaldehyde is unnecessary; the block being put directly in water and boiled as directed.

After pouring off the last change of water, the block is covered with 95 per cent alcohol. The test tube is cautiously introduced into the free flame and the alcohol boiled 3 minutes; two additional changes of alcohol being used, with the 3 minute period of boiling, just as was the case with the water. It is essential that thorough dehydration be effected by this step. That this is accomplished, can be determined only by experience, one being led to this conclusion by the appearance of the tissue.

The block is then covered with absolute alcohol, contained in a wide mouth bottle of suitable size which must be hermetically stoppered. The tightly sealed container is kept at 55° C. for  $\frac{1}{2}$  to 1 hour, depending on the size of the block.

Next, the block is placed in a solution of celloidin in ether and alcohol. The percentage of the celloidin is 6.5; of ether, 60; of alcohol, 40. It is essential that the container be hermetically sealed, and it is my practice to wire the stopper in. If the stopper should blow out in this step, the preparation will fail. The container is heated in the bath at 55° C. for  $\frac{1}{2}$  to 1 hour.

At the end of the required time, the container is removed from the bath and placed on ice in order to lower the temperature rapidly. It is necessary that the temperature of the block be the same as that of the room preliminary

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\*From the Laboratory of Clinical Pathology, Medical College of Virginia.



to the next step; if the temperature be above that of the room, the celloidin does not infiltrate properly, if it be below that of the room, bubbles are apt to form in the interior of the block. When the proper temperature has been attained, as determined by a thermometer, the block is mounted and allowed to dry in the open air for 5 to 8 minutes.

Immerse in chloroform 20 to 40 minutes and section.

By means of this method, sections may be obtained in from 4 to 5 hours. Obviously, this is a much longer time than is required for the preparation of



Fig. 1.

tissue by the frozen-section method; so that it is not my intention to propose this method as a substitute for the frozen-section method in all cases. When, however, the added time is not prohibitive, the use of this method will enable the preparation of sections which are fully as good as those which are prepared by the much more time-consuming and elaborate technic which is generally employed in cases where the frozen-section preparations are unsatisfactory.

Fig. 1 illustrates the character of the sections that may be obtained by this method; the specimen being an osteosarcoma of the breast under high power.

# The Journal of Laboratory and Clinical Medicine

VOL. VII.

NOVEMBER, 1921

No. 2

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Washington, D. C.

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## EDITORIALS

### *Botulism*

THIS review covers three recent contributions to the subject of Botulism. Orr<sup>1</sup> confines his statements to the temperatures necessary to destroy the toxin. He employed ten strains of *B. botulinus*. The toxins were prepared as follows: Into flasks there were poured 500 c.c. of a one per cent dextrose bouillon culture, to which was added 50 grams each of finely minced beef heart and marble chips. After stratification with a half-inch layer of paraffine oil these bottles were sterilized and inoculated with the several strains. After inoculation the flasks were incubated at 37° C. for three weeks. They were then filtered through Mandler diatomaceous filters and the sterile filtrates were tested for their potency. The test animals consisted of white mice weighing about 20 grams. The different strains furnished toxins, the minimum lethal dose of which varied from 0.0001 c.c. to 0.05 c.c. At 72° C., the toxins were destroyed after ten minutes; at 80° C., the seven most potent toxins were destroyed only after two minutes, while the three weaker toxins were rendered inert within thirty seconds. Orr states his conclusions as follows: "(1) The toxins produced by ten different strains of *Bacillus botulinus* were found to be comparatively thermolabile. At 80° C. they are destroyed

within from thirty seconds to five minutes, at 72° C. within from two to eighteen minutes and at 65° C. within from ten to eighty-five minutes. (2) The heating of any food material to the boiling point will destroy all traces of botulinus toxin which might be present. (3) The average temperature coefficient of the destruction of the toxins on increasing the temperature from 65° to 72° C., was found to be 5.2, while on increasing the temperature from 72° to 80° C. the average temperature coefficient was 4.2. (4) A grouping or classification of the toxins studied can scarcely be made on the basis of their resistance to heat, however the toxin produced by strain eleven (Nevin) is apparently much more resistant than any of the other toxins."

The paper by Burke, Elder and Pischel<sup>2</sup> is devoted to the treatment of botulism. These investigators have made quite an extensive study both of specific and nonspecific treatment in this disease, and in doing so they have endeavored to adapt their experimental tests to natural conditions. Before going into their work in regard to treatment, it will be of interest to note their conclusions concerning the temperature necessary for the destruction of the toxin and also the possibility of infection with this organism. Their statements on these points are condensed as follows: "(1) Spoiled foods containing gas may appear to be boiling for several minutes before the true boiling point is reached. We recommend that all suspected food be subjected to vigorous boiling for at least thirty minutes before being tasted. (2) Spoiled canned foods giving the appearance of boiling for seven minutes and subjected to actual boiling for four minutes are not safe to eat. Spoiled canned foods exposed to a temperature of 80° C. for one hour may appear to be boiling part of the time and not be safe to eat. (3) We may expect to have outbreaks of botulism following the eating of insufficiently cooked spoiled foods. (4) The heat resistance of the disease-producing power of different kinds of spoiled canned foods containing *B. botulinus* and its toxins has not been determined and probably will be found to vary. (5) There have been no recorded outbreaks of botulism in this country without a history of preserved foods having been eaten. (6) There is no evidence that infection in man ever follows the ingestion of toxin-free organisms of *B. botulinus*. (7) There is no evidence that infection in man ever follows the ingestion of the toxin and organisms of *B. botulinus*. (8) Botulism does not result from the ingestion of small numbers of toxin-free spores."

In their experimental work on nonspecific treatment, these investigators find that there are several substances which modify and diminish the action of the toxin. Some of these have a neutralizing or destructive action on the toxin. In this group are sodium hydroxid, liquid soap, and potassium permanganate. However, none of these can, at present at least, be used in the treatment of botulism for two very good reasons. In the first place, botulism is not recognized until the toxin has been absorbed and has already begun to manifest its harmful effects upon the system. There is no reason for supposing that these agents have any destructive action on the toxin after its absorption. In the second place, all of these agents, in the quantity necessary to neutralize the toxin, even in the stomach, would be in and of themselves harm-

ful. There are other substances, especially fats, such as olive oil, which afford a certain amount of protection against the toxin in experiments upon animals. The authors conclude that this protection is limited and that it is largely, possibly wholly, due to a slowing down of the rate of absorption of the toxin. These investigators appear to be hopeful and think it possible that further research may lead to the discovery of some beneficial nonspecific agent. Early students of botulism learned that, while the toxin may remain in the stomach for a long time, it is not altogether safe to try to empty the stomach, either by the administration of emetics or by the employment of a tube. The death rate among those who vomit has generally been observed to be greater than among those who do not vomit, the difference being about five per cent. Vomiting, therefore, is not regarded as a favorable symptom in botulism, and this is true whether the vomiting be natural or be artificially induced. In either case there is, on account of paralysis of the pharyngeal nerves, great danger of aspiration-pneumonia. It is suggested that a two per cent argyrol gargle might be used, but even this is, in our opinion, questionable advice. In Europe, pilocarpin has been employed in order to remove the thick, ropy mucus which accumulates in the trachea and in the throat, but this drug must be handled in botulism with so much care that we doubt very seriously its employment at all. It has been known to cause an accumulation of fluid from which pulmonary edema and death resulted. Strychnin was employed for its supposed effects upon the nervous system long before the discovery of the toxin, but, so far as we know, there is no evidence that it is of any value. Cathartics have but little effect, because the obstinate constipation which is characteristic of this disease, is due to paralysis of the walls of the intestines. The death rate from botulism in this country so far has been higher than in Germany. Whether this is due to a weaker strain in Europe or to some other cause, no one has been able to determine. The statement has been made that the toxin of botulinus is neutralized when brought into contact with nerve tissue. The California investigators studied this and state their results as follows: "Certain food substances, such as glucose, brain tissue, milk, eggs and gelatin do not neutralize the toxin of *B. botulinus* and do not protect as effectively as olive oil against the action of the toxin in subcutaneous injections." In studying the outbreak at Canton, Ohio, it was reported by Armstrong, Story and Scott that some of the guests at the fatal meal had been partaking of whiskey before dining and that these were less seriously affected than those who had not had a drink. The California investigators, experimenting upon rabbits, found that alcohol had no protective action.

In regard to specific treatment, these authors make the following statement: "Since there are at least two distinct toxins, and there is no rapid means of determining the type, it is necessary to use a polyvalent antitoxin or a Type A and a Type B antitoxin, and since there is a possibility that *B. botulinus* occasionally produces its toxin in the body, the immune serum should be bacteriolytic or bactericidal as well as antitoxic. The serum should be injected intravenously. The immune serum to be beneficial must be used



as soon as possible after the diagnosis is made. In all cases of doubtful diagnosis, all those having partaken of the suspected meal should receive immediately antitoxin treatment. The antitoxin can only neutralize the toxin and prevent further injury. It is of no value to the already damaged nervous system. The evidence we have indicates that treatment begun after the symptoms are well advanced will not, in most cases, alter the course of the disease. But we have sufficient experimental evidence to believe that some of those receiving lethal amounts of toxin can be saved by the use of antitoxin if the treatment is begun at about or before the time the symptoms appear."

While it is barely possible that a successful, or at least a beneficial, non-specific treatment may be discovered, the greatest promise apparently lies in the hope of the development of a more effective antitoxin and that provision be made for the wider distribution among health officials of the serum in order that the delay in getting it after the disease has been recognized may be greatly shortened.

Nevin was the first in this country to detect and isolate *B. botulinus*. She found this organism in home prepared cottage cheese and made a preliminary report on this finding to the American Public Health Association in September, 1915. Just recently, she has gone into this report more in detail. Since 1915 it has been demonstrated that *B. botulinus* will grow and produce its toxin not only in meat preparations, but in fruits and vegetables. So far as we know, Nevin's report is the only one in which *B. botulinus* and its poison has been found in cheese, and it must be remembered that in this case it was found in cottage cheese. No one, so far as we know, has ever reported *B. botulinus* in hard cheese or in the fromages so abundantly made and consumed both in Europe and in this country. It is worthy of note that in the long continued studies of sausage poisoning in Germany during the nineteenth century, a clear cut distinction was invariably made between botulism and other forms of food poisoning. Müller collected the literature bearing on one hundred cases of cheese poisoning and pointed out how this differs from sausage poisoning. He wrote that in cheese poisoning the duration of the disease is short and in the majority of instances there is complete recovery even without medical help within from eight to ten hours. In rare instances the untoward symptoms may continue for twenty-four hours, but death from cheese poisoning is exceedingly rare. Müller finding only one case and that of a child two years of age. In cheese poisoning the symptoms are practically confined to the gastrointestinal tract, purging is present in nearly every case and obstinate constipation is seldom or never seen. The period of incubation is short and paralytic symptoms are never in evidence. Double vision rarely appears and Ptosis palpebrarum never occurs.

The distinctive differences between poisoning with putrefactive products and botulism were formulated many years ago, and with some modifications are presented herewith, with the hope that they may aid the physician in making an early diagnosis and in distinguishing between botulism and other food poisoning:

## POISONING WITH PUTREFACTIVE PRODUCTS

- (1) Period of incubation is usually from two to four hours.
- (2) Burning in throat and stomach rare.
- (3) Dryness and redness of mouth rare.
- (4) Constipation never.
- (5) Always purging.
- (6) Difficult respiration rare.
- (7) Voice rarely affected.
- (8) Croupous cough never.
- (9) Pulse weak, scarcely perceptible, and frequently rapid (100 to 140).
- (10) Heart beat hastened, otherwise not changed.
- (11) Sensorium often cloudy; delirium and sopor.
- (12) No paralytic symptoms.
- (13) The motility of the tongue is not disturbed.
- (14) Speech not disturbed.
- (15) No difficulty in swallowing, or but seldom.
- (16) In no case is there ptosis of the upper eyelids.
- (17) Amblyopia very rare.
- (18) Double vision very rare.
- (19) Dilatation of the pupils not rare.
- (20) Ringing in the ears frequent.
- (21) The motility of the extremities not disturbed.

## BOTULISM

- (1) Period of incubation is usually from twelve to twenty-four hours.
- (2) Burning in throat and stomach frequent.
- (3) Dryness and redness of mouth constant.
- (4) Constipation always (although occasionally preceded by temporary diarrhea).
- (5) Purging absent or present only in beginning.
- (6) Difficult respiration frequently observed.
- (7) Voice frequently affected.
- (8) Croupous cough very often.
- (9) Pulse weak, scarcely perceptible, often slow (50 to 60).
- (10) Heart beat often not perceptible, frequently slow.
- (11) Sensorium always clear even to death.
- (12) Paralytic symptoms frequent.
- (13) The motility of the tongue is often disturbed and movements of the tongue are difficult.
- (14) Speech frequently disturbed.
- (15) Difficulty in swallowing frequent.
- (16) Ptosis of the upper eyelids is common.
- (17) Amblyopia very frequent.
- (18) Double vision very frequent.
- (19) Dilatation of the pupils very frequent.
- (20) Ringing in the ears rare.
- (21) The motility of the extremities greatly weakened.

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- <sup>1</sup>Jour. Med. Res., 1921, xlii, 127.  
<sup>2</sup>Arch. Int. Med., 1921, xxvii, 265.  
<sup>3</sup>Jour. Infect. Dis., 1921, xxviii, 226.

—V. C. V.

### *Another German Cure for Tuberculosis*

AT THE meeting of the International Medical Congress in Berlin in 1890, Robert Koch, the discoverer of the tubercle bacillus, made the sensational announcement that he had found "a substance which when injected into a guinea pig renders this animal insusceptible to inoculation of the tuberculosis virus. The same substance in guinea pigs already afflicted with advanced and

generalized tuberculosis, brings about a complete arrest of the development of the disease, although the animal organism does not suffer the least harmful effect under the influence of the remedy."

This announcement threw the whole world, both lay and medical, into a state of violent exaltation. Specialists in the treatment and cure of this disease flocked to Berlin and jostled one another in their efforts to secure this remedy, more precious than gold. Fabulous sums were offered for this new elixir of life. Patients on their death beds in distant parts of the earth sent messengers to Berlin to secure the magic remedy. For months Koch gave no definite information concerning this wonderful preparation. He disclosed neither its source nor its chemical composition. Finally in January, 1901, he made the following statement: "The remedy with which we aimed to cure tuberculosis is a glycerin extract of pure cultures of tubercle bacilli."

Later it was found that Koch's tuberculin was a concentrated glycerin bouillon culture from which the unbroken bacilli had been removed by filtration. Thirty years have elapsed since Koch made his remarkable statement to the Medical Congress at Berlin. No evidence has been secured during these years which gives any adequate justification of the claims that he made at that time. Koch's announcement was made in August, 1890. In November of the same year, Trudeau published in the Medical Record, protocols showing that he had used dead tubercle bacilli and filtered and sterilized cultures of tubercle bacilli in attempts to immunize animals and that his results had been uniformly negative; in other words, at the time Koch made his announcement, Trudeau, from his little laboratory at Saranac, announced to the world the failure of tuberculin to immunize animals.

In 1913 Dr. Friedrich Franz Friedmann came to New York, took up his residence at the Waldorf-Astoria, and announced that he was prepared to cure tuberculosis. Again there was great excitement, and this distinguished German savant exchanged his turtle tubercle bacilli for American gold. There is no evidence, so far as we know, that his treatment did any patient positive and lasting good. Fortunately, in 1913 the American people were not so easily stampeded as they were in 1891. The American Congress had, in 1902, enacted a law providing for the supervision of the preparation and sale of viruses, serums, and toxins in interstate traffic. This Act, in part, reads as follows: "No person shall sell, barter, or exchange, or offer for sale, barter, or exchange in the District of Columbia, or send, carry, or bring for sale, barter, or exchange from any state, territory, or the District of Columbia into any state, territory, or the District of Columbia, or from any *foreign country* into the United States, \* \* \* any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention and cure of diseases of man, unless (a) such virus, serum, toxin, antitoxin, or product has been propagated and prepared at an establishment holding unsuspended and unrevoked license, issued by the Secretary of the Treasury as hereinafter authorized, etc."

Notwithstanding the rigidity of this law, every courtesy was extended to Dr. Friedmann and he was asked to make known the nature, the preparation and the physiologic or curative action of his new product. On the receipt of this request, Dr. Friedmann promised to supply a representative of the U. S.

Public Health Service a culture of this organism, but he stated that it would be of no use to the officer or to any one else for a proper study unless the person receiving it had been instructed by him during a period of from six to eight weeks as to its proper manner of preparation and use. He further stated that he would permit an investigation, but that the person charged with it should have a training under him. In due time the Surgeon General of the U. S. Public Health Service obtained samples of Dr. Friedmann's preparation, a thorough investigation of his claims was made, and finally reported upon by the Surgeon General.<sup>1</sup> The conclusions reached by the commission of the Public Health Service are stated as follows: "The claims made by Dr. Friedmann for his method of treating tubercular infections are, in brief, that, by means of injections of a living acid-fast organism, harmless of itself, he is able to cure cases of tuberculosis, pulmonary or otherwise, which have not already advanced to that hopeless stage where death is imminent. From the manner of presenting these claims and from the fact that successes only and not failures are reported, the reader of these claims is bound to assume that such results are the rule; in other words, that a sovereign remedy for tuberculosis has at length been discovered and incidentally that a method has been devised for the administration of living acid-fast organisms which avoids abscess formation, a complication which has hitherto limited their employment.

"The results of the investigation here reported do not confirm the claims made by Dr. Friedmann. We find, in brief, that the preparation used by him is not strictly devoid of dangerous properties of itself, still less so when injected into tuberculous subjects; that the favorable influencing of tuberculosis processes by his method is certainly not the rule, and that if we are to ascribe to the Friedmann treatment the improvement noted in a few cases, we are equally bound to impute to it the serious retrogression observed in other cases; and finally that the phenomenon of abscess formation has not been avoided by Dr. Friedmann's methods.

"We find that the organism used by Dr. Friedmann differs in important cultural characteristics from any heretofore recognized tubercle bacillus.

"The subcutaneous and intramuscular inoculation of animals with the Friedmann organism caused the formation of abscess in over twenty-five per cent of the animals treated.

"The treatment of animals with the Friedmann organism—rabbits and guinea pigs—either before or subsequent to infection with virulent tubercle bacilli, is followed, as a rule by an increased susceptibility to the disease.

"Inoculation of monkeys with the Friedmann culture did not show either curative or protective action in those animals against tuberculosis.

"The claim of Dr. F. F. Friedmann to have originated a specific cure for tuberculosis is not substantiated by our investigation.

"The claim of Dr. F. F. Friedmann that the inoculation of persons and animals with his organism is without harmful possibilities is disproved."

A few days ago we received a little book,<sup>2</sup> which we have read from cover

<sup>1</sup>The Friedmann Treatment for Tuberculosis, Hygienic Laboratory Bulletin No. 99, October, 1914.

<sup>2</sup>Tuberculosis of Children, Its Diagnosis and Treatment, by Professor Dr. Hans Much, Director of the Department for the Science of Immunity and for the Research of Tuberculosis at the University of Hamburg, Germany; translated by Dr. Max Rothschild, Medical Director of the California Sanatorium for the Treatment of Tuberculosis, San Francisco and Belmont, Calif., New York, Macmillan Company, 1921.



to cover with much interest. The translator says: "The skeptical attitude of the medical world respecting new discoveries relating to the treatment of tuberculosis, is, in a sense, justified by the many disappointments which have been experienced during the last twenty years." Then the translator goes on to say that when a method is presented and supported by authentic research we should lay aside our prejudices long enough for a fair and unbiased consideration.

The name of the distinguished German author is well known, especially to students of tuberculosis. The first chapters are semipopular, pleasingly written, and on the whole, truthful. We recall the shades of Pasteur when our author informs us that the discovery that the anthrax bacillus may be reduced in virulence and used as a vaccine, was made by Behring. Much goes on to tell us why Koch's tuberculin and Friedmann's bacillus failed to cure tuberculosis. Then the real kernel in the book comes out, and we are informed: "With the aid of these deductions and proofs we have been able to locate the real origin of tuberculosis and to wage our fight in its greatest stronghold, childhood." After reading such quotations as the above, we turn each new page with tremulous expectation. We expect to find an exact scientific description of the curative agent which is to free childhood of the burden imposed upon it by tuberculosis. We find, however, that we must content ourselves with the statement that the wonderful discovery consists of "partigens," which means partial antigens. How these partigens are prepared seems to be known only to the discoverer. Minute directions are given for their administration, and we are told that these partigens can be purchased from Kalle & Company, Biebrich-on-Rhine.

After reading the book we must admit that it leaves but one impression upon us, and that is, that the purpose of its writing is to sell the "partigen." There are no details concerning the preparation of this curative body, no record of animal experimentation, no experimental protocols, and apparently we are asked to accept the statements of the distinguished author without asking any questions. Here is a book published by one of the most reputable and best known publishing houses in the English speaking world and translated by an American physician, apparently in charge of a state sanatorium. We are asking ourselves, whatever we may think or surmise concerning the purpose in writing the book, whether any responsibility lies with the translator and the publisher.

—V. C. V.

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### *Eosinophilia in Parasitic Infections*

AN INCREASE in the absolute and in the relative number of eosinophiles in the circulating blood usually accompanies hookworm infection. It is generally stated that the eosinophilia in this condition ranges from 15 to 25 or even 30 per cent. A similar increase is seen in infection of the gastrointestinal tract with other parasites such as hydatid cyst and trichina. In ankylostomiasis the development of eosinophilia begins relatively soon after infection. In two cases of experimental infection Boycott found that at the end of three

weeks the eosinophiles had increased, in the first from 2 to 14 per cent, and in the second, from 3.4 to 24 per cent. According to various investigators an increase above 8 per cent is present in from 65 to 94 per cent of all hookworm patients. It has been found that the younger and more robust individuals react with a higher eosinophilia than do the aged or anemic. Those recently infected, without anemia, as a rule show a higher eosinophilia, Boycott has observed in such a patient an eosinophile count as high as 72.7 per cent. The absence of eosinophilia in heavily infected anemic patients is of grave prognosis.

Following removal of the parasite by appropriate treatment, very decided changes occur in the blood picture. In those with high eosinophilia before treatment, the count gradually falls, after treatment, to normal. On the other hand, in anemic individuals having a low eosinophilia or none at all, removal of the parasites frequently results in an increase in the absolute and relative number of eosinophiles. Increases of as much as 35 per cent following treatment have been recorded.

This post-therapeutic increase continues for from one to six weeks and then gradually falls away. However, the complete return to normal is exceedingly slow. It is a question in some cases whether there is ever a complete return. Lamierre and Lantuéjoul found that an eosinophilia of 8 per cent persisted as long as five months after treatment while Boycott found 11 per cent in a patient three years after apparently complete cure.

The increase in the eosinophile count after treatment parallels to some extent the increase in the red cell count and hemoglobin. After a time the former ceases to increase and diminishes gradually while the hemoglobin and red count continue to rise.

Lamierre and Lantuéjoul point out that the increase in eosinophiles after treatment is entirely different from the post-therapeutic reaction in hydatid cyst infection. After recovery from the latter the eosinophiles immediately decrease. Chauffard and Boidin report a case in which the count fell within eight hours after the removal of an hydatid cyst, from 38 per cent to 1 per cent. In this disease a persistence of eosinophilia after removal indicates either that there is another cyst present or that the one operated on was incompletely removed. This is not the case with ankylostomiasis. In the latter the increase in eosinophiles following treatment may occur even though all parasites are proved to have been removed. It is true that in 26 per cent of cases more than 2 doses of anthelmintic are necessary for complete removal. In Egypt Sandwirth found it necessary to use even as high as 11 doses for satisfactory results. The absence of ova in the stools on microscopic examination does not prove that the patient has been cured. According to Brüns and Mackel, hookworm larvæ may be cultivated from fecal matter which is apparently free from ova on routine microscopic examination. Nevertheless the eosinophile increase cannot be satisfactorily explained on the grounds of residual infection. It has been quite amply demonstrated in individuals who have been watched over long periods and who have remained free from subsequent evidences of disease, that this post-therapeutic eosinophilia exists.

Lamierre and Lantuéjoul have offered an explanatory hypothesis. They point out that individuals infected with hookworm have at necropsy a duodenal wall infiltrated with enormous numbers of eosinophiles. These cellular elements may have been formed in that locality or more probably they are true eosinophiles coming from the bone marrow by way of the blood stream.

Weinberg and Seguin have experimented with intestinal parasites in horses. They find that the local accumulation will not occur in the region of fixation of the parasites unless the animals possess eosinophiles in the circulating blood. The local eosinophilia in the wall of the intestine is proportional to the degree of eosinophilia in the circulating blood. Injection of an eosinophilotactic substance causes an almost immediate lowering of the eosinophile count in the circulating blood coincident with a local increase at the site of injection. These facts point toward hematogenous origin. The latter authors explain the almost immediate decrease in circulating eosinophiles following hydatid cyst removal as due to the necessary spilling of small quantities of fluid during removal. The eosinophilotactic action of this fluid draws the cells from the blood, to the site of operation.

Lamierre and Lantuéjoul suggest that the increase after removal of the hookworm may be due to the removal of the eosinophilotactic substance. They conceive of the bone marrow as having been acted upon by a poison absorbed from the region of the duodenum and which stimulates the production of eosinophiles. With the loss of the eosinophilotactic substance from the duodenum and with continued increased output from the marrow, eosinophiles accumulate in the blood stream. These authors believe that the hypergenesis in the bone marrow persists for some time, and that the post-therapeutic increase is due to disturbed balance between the number of eosinophiles in the blood and those drawn to the duodenal wall.

If, on the contrary, the eosinophilia is due to the local formation of eosinophilic cells in the duodenal wall, and the increased count in the blood is due to overflow from this area, these authors believe that the increase would be explained by the therapeutic removal of those substances which tend to retain these cells *in situ*.

Eosinophilia is usually associated with the presence in the body, or absorption into the body of protein substances or protein split products. It is a phenomenon of anaphylaxis, of serum sickness and of bronchial asthma. Where the foreign protein has a localized distribution, eosinophiles will usually be found in such localities. This is true of the lungs in bronchial asthma, and of encysted embryos in trichinosis. Opie has shown that in experimental trichinosis the bone marrow is usually increased in extent and shows large numbers of mature and immature eosinophilic cells. In very severe infection these cells may show evidences of degeneration. He finds that the increase in eosinophiles in the circulating blood does not begin until the parasites have begun to pass to the muscles. At this time proliferation in the bone marrow becomes quite active. Opie has shown that in experimental animals infected with trichina, eosinophiles move from the bone marrow into the blood stream and from the latter to the sites of inoculation. Although they behave in

this respect somewhat similarly to neutrophilic leucocytes, they rarely show active phagocytosis.

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—W. T. V.

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*Acne Vulgaris*

**A**CNE vulgaris is one of the commonest skin affections, so common that it has been estimated that 40 per cent of the population suffer from it. By most physicians it is looked upon either as insignificant or as a nuisance as the case may be, though by some it serves, because of its intractability, as a mere source of income. To the ambitious practitioner it is a *bête noir*.

It is a disease which has, so far as is known, no mortality. It does not incapacitate for work, and yet it is often the cause of unpleasant social situations, and not infrequently it casts a cloud over an otherwise bright and happy career.

Patients looking perfectly healthy except for the local lesions, ask medical advice for the cure of the disease for social rather than for physiologic reasons. If the blemishes one sees on the faces of passers-by on our main thoroughfares is any indication of therapeutic success, then the results are lamentable. Nevertheless the disease is almost without exception readily and promptly cured. One measure depends upon the use of vaccines preferably autogenous ones. There are cases, a minority one would say, which respond very perfectly to such therapy. But it is often a difficult matter to procure a vaccine. In many cases of acne it is almost impossible to isolate the *B. acnis* in pure culture, and its growth is slow when it is isolated. Nevertheless it seems true that certain cases are benefited by either a mixed autogenous vaccine, composed of the staphylococcus so frequently a secondary invader of the acne lesion, and the acne bacillus; or one composed of the staphylococcus alone. Cases responding promptly to this latter agent are probably not true acne. Generally speaking, however, the vaccine treatment has been discouraging, as Fox demonstrated in his experiments carried out at Cornell University.

But better than vaccines and infinitely less troublesome to the patient, is x-ray therapy. LaFevre, in a report of his experience with this method, covering a period of ten years, and several hundred cases, says that there were but a few individuals who did not entirely clear up, and that these were improved. His results appear to be the exact reverse of those of Fox.

The writer has yet to see a case which has not responded satisfactorily to this form of treatment, but he urges that the amount of the ray used must be judged carefully for each case, after ascertaining what local treatment has been used previously. An erythema should *just* be avoided. With a careful technic not more than two treatments are required as a rule, and very rarely are more than three treatments needed.



Recent advances in radiotherapy are so numerous and rapid that few physicians know them all. Those who have attempted to keep up find it difficult to choose a conservative middle course between the overenthusiastic and the skeptic. The arguments of one appear to be about as convincing as those of the other. It is for this reason that we think it of value to call attention to the successful methods which bear the test of practice, and to suggest that, in the case of *aene vulgaris*, radiotherapy be tried as the first remedy and not as the final resort.

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—C. C. B. (P. G. W.)

## CORRESPONDENCE

### The Constitution of Arsphenamine

*To the Editor:*

In a review of the "Chemotherapy of Organic Arsenicals and the Related Physical Phenomena" by C. N. Myers, Jour. Lab. Clin. Med., vol. vii, No. 1, p. 23, I am erroneously quoted as stating, in substance, that the volatile matter of commercial arsphenamine is entirely methyl alcohol of crystallization and that it is this methyl alcohol that is responsible for the untoward reactions that follow the use of the drug intravenously.

In the Jour. Lab. Clin. Med., iv, No. 4, under the caption "Notes on the Decomposition of Arsphenamine" I stated that "Commercial Arsphenamine is a mixture of dihydroxydiaminoarsenobenzenedihydrochloride, its oxidation products, mineral impurities chiefly sulphur, *water, methyl alcohol* and *ether*." The volatile impurities on three samples of arsphenamine that had been found especially toxic were stated as amounting to 7.76, 4.60 and 6.19 per cent respectively, and of this 20.33 and 23 per cent, respectively, was accounted alkoxyl, figured as methoxyl. This would, of course, include ether, alcohol, and acetone. I have at no time used the term "water of crystallization" or "methyl alcohol of crystallization," indeed I stated that "the uneven loss of weight \* \* \* argues against the presence of water of crystallization and suggests rather hygroscopic moisture or water of occlusion" and that "the methyl alcohol and ether is probably held in the same way." My methods of work and the figures obtained were published at that time in detail. Myers states (p. 23, loc. cit.) that in some samples of arsphenamine, a maximum of 2 per cent of methyl alcohol was recovered. This amount is greater than the amount of alcohol, ether, and acetone combined that I reported for any of the samples of arsphenamine I examined, and these samples were all selected because of their high toxicity. Myers' observations on the methyl alcohol

content are therefore largely confirmatory of my work done three years before.

The possibility of this amount of methyl alcohol proving toxic I have never considered. I have, however, pointed out that by esterification with arsenic under the low pressure of the ampoule, a highly toxic alkyl arsin might be formed and greatly increase the toxicity of the solution. The fact that solutions made with hot water, and solutions that have stood before use are less toxic than they would otherwise be, certainly favors the assumption that a volatile impurity is present. On a statistical basis I have found solutions perforated with a brisk current of hydrogen for one minute before use about 15 per cent less toxic to a parallel series of ten rats than when used immediately.

Reactions with any of the arsphenamines have been unknown in this clinic the past year, during which over 500 full doses have been intravenously administered. Three years ago when the manufacture of the drug in America was still in an experimental stage; untoward results were obtained with distressing frequency. This change to my mind means an improvement in the drug itself, be it chemical or physical.

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# *The Journal of Laboratory and Clinical Medicine*

VOL. VII.

ST. LOUIS, DECEMBER, 1921

No. 3

## ORIGINAL ARTICLES

### A NOTE ON THE BLOOD CHLORIDES IN MERCURIC CHLORIDE NEPHROSIS\*

BY JOHN A. KILLIAN, PH.D., NEW YORK CITY

COMPARATIVE studies of the composition of the blood and urine in a case of mercurial poisoning were reported in 1915 by Myers and Fine<sup>1</sup> who observed that the accumulation of nonprotein and urea nitrogen, of the uric acid and of the creatinine in the blood was coincident with a diminished output of these compounds in the urine. After 5 days of anuria, the nonprotein nitrogen of the blood had risen to 338, the urea nitrogen to 240, the uric acid to 15, and the creatinine to 33 mg. per 100 c.c. After decapsulation of the kidneys a temporary improvement of renal function was noted with a decrease of the concentration of these nitrogenous constituents in the blood, and an increased output in the urine. A subsequent decline of kidney activity, however, resulted in death. The authors emphasized the diagnostic and prognostic significance of the chemical analysis of the blood in mercuric chloride nephrosis, and in chronic nephritis. A review of the literature since that time reveals abundant data upon changes in the concentration of the nitrogenous components of the blood following mercuric chloride poisoning but comparatively little attention has been accorded other equally important constituents. In a nonfatal and less severe case, described by Cohen and Bernhard,<sup>2</sup> the clinical improvement was preceded by a marked decrease in the nonprotein and urea nitrogen, and of the creatinine of the blood after the introduction of large quantities of alkaline fluid by mouth, rectum, and under the skin. Similar observations are recorded by Rosenbloom,<sup>3</sup> Underhill<sup>4</sup> and Campbell.<sup>5</sup>

It is significant that in mercuric chloride nephrosis despite the severe impairment of renal function indicated by the marked retention of the nitrogenous waste products, edema is generally absent. In other types of nephrosis,

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however, an anasarca usually develops consequent to an impairment of chloride excretion. Campbell drew attention to this fact in his studies on a fatal case of mercuric chloride poisoning. Here the chlorides of the blood plasma had dropped within 8 days from 0.666 to 0.521 per cent, whereas the nonprotein nitrogen rose from 170 to 319 mg. per 100 c.c., and the sugar from 0.20 to 0.46 per cent. That the retention of the nonprotein nitrogenous components of the blood in this type of kidney injury is not accompanied by an increase in the blood chlorides is further evident from the work of Lewis and Rivers.<sup>6</sup> It was noted by them that in a case of bichloride of mercury poisoning, after the ingestion of large quantities of fluid, the plasma chlorides reached their lowest level (0.385 per cent) when the nonprotein nitrogen was at its maximum concentration (178 mg. per 100 c.c.). After 14 days of treatment leading to recovery the nonprotein nitrogen had been decreased to 22 mg. but the plasma chlorides had returned to 0.595 per cent. In like manner, Woods<sup>7</sup> has reported a low concentration of the chlorides of the whole blood (0.342 per cent) coincident with a pronounced retention of the nitrogenous waste products in one case of bichloride poisoning, and in several cases of chronic nephritis. Although a high blood chloride was usually found in the cases of severe nephritis studied by Myers,<sup>8</sup> in one instance a restriction of the chlorides of the diet and a large fluid intake produced a drop in the concentration of the chlorides of the whole blood from 0.594 to 0.394 per cent, but during the same period the nitrogenous waste products were markedly increased.

MacNider<sup>9</sup> has emphasized the influence of the disturbance of the acid-base equilibrium of the blood in the production of the kidney injury in mercurial poisoning. He noted that in the animals surviving the gastroenteritis following poisoning with mercuric chloride the injury to the kidney was constantly associated with the development of an acid intoxication. The extent of the impairment of renal function bore no relation to the elimination of the mercury in the urine, and was not due to the action of the metal upon the kidney tissue. However, the progress of the kidney insufficiency kept pace with the drop in the alkali reserve of the blood and the excretion of ketone bodies. A restoration of the normal acid-base equilibrium of the blood was accompanied by a return to normal of the functional capacity of the kidneys.

The object of the present communication is to present the results of observations on two nonfatal cases of mercurial poisoning. It is believed that these results, though not entirely new, may be of interest in view of the points previously discussed. These studies comprise analysis of the blood, specimens of which were obtained at frequent intervals, for urea nitrogen, uric acid, creatinine, chlorides (of the whole blood), sugar, and the carbon dioxide combining power. The methods of analysis described by Myers<sup>10</sup> were employed in all instances. Daily routine examinations of the urine were made. The total fluid intake and output were measured each day, and the stomach washings and specimens of urine were examined for mercury by the method of Vogel and Lee<sup>11</sup> until these tests proved the absence of the metal. Significant clinical changes are also noted.



TABLE I

CHEMICAL BLOOD FINDINGS IN BICHLORIDE POISONING WITH SPECIAL REFERENCE TO THE CHLORIDES

CASE	DATE 1921	CHLORIDES AS NaCl	URIC ACID	UREA N	CREAT- ININE	CO <sub>2</sub> COMBINING POWER
		per cent	mg.	to	100 c.c.	c.c. to 100
1.—R. S., female, aged 19, 15 grains HgCl <sub>2</sub> taken	May 9	0.495		22		36
	" 11	0.338	8.3	90	10.7	
	" 13	0.388		88	9.1	30
	" 15	0.114		98	12.0	27
	" 17	0.207		73	12.0	
	" 19	0.250	8.0	75	9.3	
	" 21	0.363	2.8	91	7.8	81
	" 27	0.500	1.0	18	4.4	61
	June 6	0.525	1.9	13	2.2	
2.—E. F., female, aged 20, 15 grains HgCl <sub>2</sub> taken	May 17	0.382	9.6	146	14.5	36
	" 23	0.410	10.9	188	15.8	25
	June 11	0.563	1.5	11	2.2	

Case 1.—Admitted to Lincoln Hospital evening of May 7. Four to five liters of alkaline fluid introduced as gastric lavage, colonic irrigations and enemas; and hot packs given from May 7 to 27. In addition to this, 1000 c.c. of 0.7 per cent NaCl was introduced hypodermically daily from May 16 to 25. No anuria and no edema. Phenolsulphonephthalein excreted in traces only until May 25; at this time output was 25 per cent, and rose to 45 per cent on June 13. Diet fluid and salt-free. Clinical improvement noted about May 20. Discharged from hospital cured June 19.

Case 2.—Admitted to Harlem Hospital May 11. Gastric lavage with alkaline fluids for 4 days. One thousand c.c. Fischer's solution given intravenously daily from May 11 to 14. High colonic irrigations, large quantities of fluids by mouth, and hot packs given daily until June 11. Diet fluid and salt-free. Clinical improvement noted about June 8. Discharged from hospital cured June 15.

## DISCUSSION

Both cases present evidence of severe nitrogen retention, which is more marked in Case 2 than in Case 1. It will be noted in Case 1 that the retention of urea nitrogen began about 36 hours after the ingestion of the mercuric chloride. In the second case no specimens of blood were obtained until 72 hours after the administration of the poison, and at this time the nonprotein nitrogenous constituents of the blood had already reached a very high level. Within 8 days the urea nitrogen in Case 1 had been increased steadily to 98, and the creatinine to 12 mg. per 100 c.c. Following this, a gradual clinical improvement was noted, and this clinical improvement parallels the decrease in the uric acid, urea nitrogen and creatinine of the blood. It is worthy of note that the decrease in the uric acid precedes the drop of both the urea nitrogen and of the creatinine. The same may be said to be true of Case 2. In this particular, our findings are in close accord with the observations of Cohen and Bernhard, Rosenbloom, Underhill, and Campbell. These two patients, and as well the cases described by the authors mentioned, received large quantities of fluid by mouth, rectum, and hypodermically, and in Case 2 intravenously. The fatal case described by Myers and Fine manifested a more marked retention of the nitrogenous waste products than any of patients referred to. In this case, however, there was no attempt to increase the fluid intake, which fact may account for the greater retention of nitrogen.

Since Myers and Short<sup>12</sup> have pointed out that, as an index of any significant change in the concentration of the blood chlorides, a determination of the chlorides of the whole blood is more trustworthy than a determination of the chlorides of the plasma, our studies have been confined to the chlorides of the whole blood. These authors have placed the normal chlorides of whole blood between 0.45-0.52 per cent. A remarkable decrease in the blood chlorides from 0.495 to 0.114 per cent is noted in Case 1 within the first 8 days of treatment, but the nitrogenous constituents however, rose to their maximum concentration. During this period the patient was receiving per day a total of 4.5 liters of alkaline fluids containing no chlorides. For the succeeding 8 days a hypodermoclysis of 1000 c.c. of 0.7 per cent sodium chloride solution was given daily. There follows a gradual rise in the concentration of blood chlorides to 0.525 per cent. Simultaneous with this increase in the chlorides, there is a diminution of the nitrogenous constituents of the blood, keeping pace with the observed clinical improvement. In like manner in Case 2 with a pronounced retention of nitrogen, low figures were obtained for the blood chlorides. This patient, however, at no time manifested as marked a decrease in chlorides as Case 1, which may be due to the fact that Case 2 had received for the first 3 days of treatment, 1000 c.c. of Fischer's solution intravenously. Here again, with a restoration of the normal values for the nitrogenous waste products, the blood chlorides return to an unusually high level, 0.563 per cent. The work of Lewis and Rivers demonstrates that the reduction of the blood chlorides in mercurial poisoning cannot be explained by a flushing out of the salts in the urine after the ingestion of large quantities of fluid. On the contrary they found low blood chlorides coincident with a diminished excretion of salt, and as the chlorides of the blood rose there was a proportionate increase in their excretion in the urine. Myers and Fine also observed a subnormal excretion of chlorides associated with comparatively low blood chlorides. Furthermore, there was not sufficient variation in the hemoglobin, or in the enumeration of the blood cells to warrant attributing the reduction in the chlorides to a dilution of the blood.

McLean<sup>13</sup> has reported somewhat analogous findings for the chlorides of the blood and urine in lobar pneumonia. During the active stage of this disease he finds the failure of the kidney to excrete chlorides to be associated with a subnormal concentration of chlorides in the blood plasma. At the crisis the plasma chlorides rose abruptly and excretion began and continued in proportion to the concentration in the plasma. It would appear, then, that in mercuric chloride nephrosis, as in lobar pneumonia, the diminished excretion of chlorides must be ascribed to a decrease of the blood chlorides below the threshold point, and with a rise of the chlorides the amount excreted in the urine proportionately increases. The mechanism of chloride retention with a simultaneous decrease of the chloride concentration in the blood in mercuric chloride nephrosis is at the present time an obscure point.

At the time of the greatest impairment of kidney function, there was observed a marked decrease in the carbon dioxide combining power of the blood plasma. In Case 1 the carbon dioxide combining power dropped to 27,

and in Case 2 to 25 volumes per cent. A rise in alkali reserve of the blood plasma in the first instance followed the use of alkaline fluids, in gastric lavage, colonic irrigations and enemas. This rise accompanied the improvement of renal function. These findings are of interest in view of MacNider's contention, but whether the restoration of the normal functional capacity of kidneys is to be explained by the protecting action of the alkalies cannot be decided by our data.

It is also worthy of note that in both cases no evidence of edema was presented at any time. The volumes of urine excreted in 24 hours were moderately increased, with specific gravities ranging from 1008 to 1022. In both instances, occasionally sugar was detected in the urine with Benedict's qualitative reagent although the blood sugar did not rise above 0.114 per cent. Glycosuria is not an unusual finding in certain types of kidney disease particularly after poisoning by metallic salts. Myers and Kast<sup>14</sup> have reported glycosuria in 3 cases of chronic nephritis with but slight nitrogen retention, but with edema and constant proteinuria. In one instance there was a normal blood sugar, and in the other two but slight hyperglycemias. The glycosuria in the latter was found to be independent of the hyperglycemia. Cohen and Bernhard, likewise, noted a glycosuria of 1.5 per cent with a hypoglycemia. An examination of the ocular fundi revealed nothing abnormal.

#### SUMMARY

Two nonfatal cases of mercuric chloride poisoning are reported. As the impairment of renal function due to kidney injury progressed, a diminution of concentration of the chlorides of the whole blood was noted. A return to normal of the functional capacity of the kidneys was accompanied by an increase in the blood chlorides.

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# CLINICAL DIAGNOSIS BY THE AID OF VISCOSIMETRY OF THE BLOOD AND THE SERUM WITH SPECIAL REFERENCE TO THE VISCOSIMETER OF W. R. HESS

BY M. E. BIRCHER, M.D., ROCHESTER, MINNESOTA\*

THE determination of the viscosity of the blood in the study of hemodynamics (physiology of the circulation) and in general diagnosis has not received the attention of clinicians because of the lack of a practical manner of its determination and the difficulty in the interpretation of results. During the last twenty years approximately 200 papers have been published on the subject, most of them after the introduction in 1906 of the viscosimeter of Hess,<sup>20</sup> the only apparatus which fulfills all the requirements for a practical and accurate test. But contradictions and uncertainty concerning the application of the test prevailed until recently a practical clinical basis was established for it by Naegeli.

English and American investigators have contributed about twenty articles on the viscosity of the blood: in this country Burton-Opitz has been one of the most industrious pioneers. His valuable contribution is too purely physiologic in nature for clinical application, although his results merit an attentive study, especially since the apparatus of Hess replaces his more complicated technique. His conclusion may be quoted here as it was presented to the American medical profession in 1911 to emphasize the value of a broader consideration of the viscosity factor in circulation: "The viscosity represents an independent dynamic power which, although slight normally, will become overwhelmingly great, if other factors favor its development."<sup>15</sup> McCaskey, in 1908, presented a very simple and effective viscosimeter, but the duration of the determination extends into the phase of coagulation. The best English review on the manifold questions related to the viscosity of blood was published in 1911 by Allbutt who gave a broad conception of the relation of viscosity to the physiology of circulation, discussed all the known facts pointing to the difficulty of interpretation, and predicted a more general recognition of its significance in the future. Recently Langstroth studied the viscosity of the blood after its withdrawal from the body. These studies are of theoretic value only, corroborating old findings and presenting many errors because the thorough previous investigations are not considered carefully enough.

The discussion of theoretic problems concerning the viscosity of the blood will be limited in this review on account of the space which it seems advisable to give to more practical details, in order that any practitioner or clinician may have a basis for the application of viscosimetry, and may successfully carry it out in his laboratory and at the bedside.

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## VISCOSIMETER OF HESS

The viscosimeter of Hess is not only the simplest, but also the most reliable apparatus, especially for high values, since it eliminates many technical errors. Hess devoted several critical studies to his own apparatus, and was able to give clear scientific proof of the principles involved in its mechanism. Independently Kagan, of Sahli's Clinic, in an elaborate criticism of the different viscosimeters presented a satisfactory answer to all the possible objections to Hess's instrument.

The apparatus is based on the law of Poiseuille,\* according to which fluids, at equal temperature and equal pressure, passing through capillary tubes of equal caliber, vary in their rate of flow in direct proportion to their internal

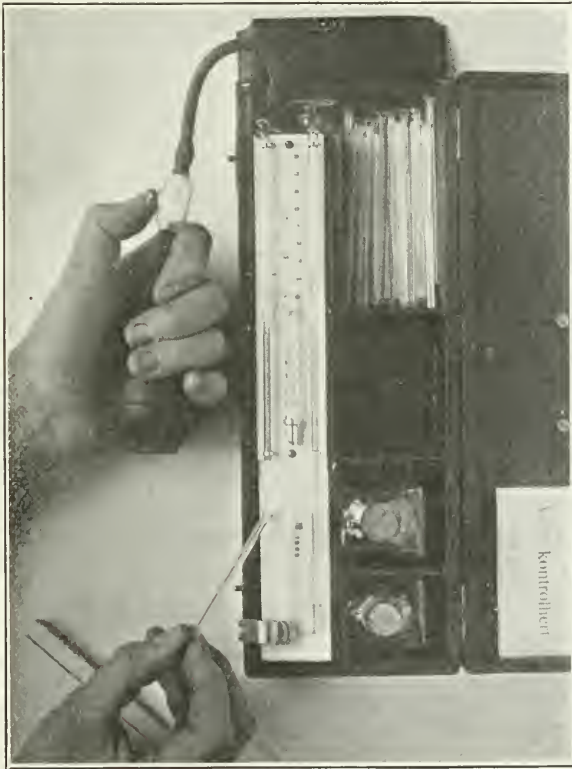


Fig. 1.—Viscosimeter of W. R. Hess. The picture illustrates the handling of the apparatus the moment the blood drop is drawn in.

friction, that is, to their viscosity. The law can be applied only when liquids are driven through the capillaries by a certain pressure. This pressure should be moderate so as to obtain a gentle flow of the liquid, but it must be greater than the force of gravity. The latter is employed in many other instruments and its incompatibility with the law of Poiseuille is the chief objection against the well-known apparatus of Determann (Fig. 1).

By the aid of a strong rubber bulb, blood and distilled water are drawn simultaneously under the same pressure through two capillaries. If the cap-

\*Poiseuille was the first to study the problem of viscosity and published his fundamental law in 1847.

illaries are of equal length and lumen, and if the temperature, pressure, and time are the same for both capillaries, the flow through volume of two liquids is inversely proportional to the viscosity. The volume is measured by a graduated glass tube, and the volume of water taken as the standard. The flow through volume of any fluid more viscous than water will be smaller than that of water, or the flow through volume of water will be just so much larger as the tested fluid is more viscous. If blood is sucked from 0 to 1 the water column reaches a point in the graduated tube which indicates directly how much farther the water was flowing under the same conditions. At this point a reading is taken. This reading is exactly the viscosity of the blood, compared with water, and is therefore relative.

The viscosity of a fluid is expressed in viscosimetric units of distilled water at a temperature of 20° C. Within certain limits it is not necessary to consider the temperature, although this may easily be done. The temperature recorded by the thermometer which is placed between the capillaries, should be between 17°C. and 23°C. Tests made at these temperatures need no correction because the error will not exceed  $\pm 3$  per cent when 20° C. is taken for the standard. Any deviation from these limits is corrected by adding for every degree

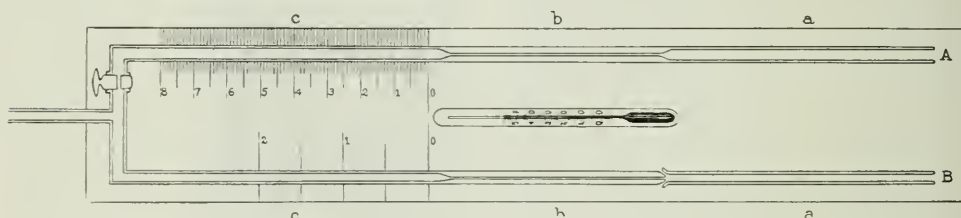


Fig. 2.—Diagram of the viscosimeter of Hess. The flow through volumes of water and blood are measured in the respective tubes (c.c.). The two capillaries (b.b.) are of exactly the same size, but the water tube for measurement is slightly larger than the glass tube as distinctly shown in the diagram. This fact explains why the units of the water tube are smaller than those of the blood tube, but it must be understood that the volumes are exactly the same.

above 20° C., 0.8 per cent of the reading, and by subtracting 0.8 per cent for every degree below 20° C. For laboratory research Hess has devised a larger model with a water cylinder, which makes it possible to carry out series of determinations at constant temperatures.

#### DESCRIPTION OF PARTS

I. The viscosimeter is composed of the following parts (Figs. 1 and 2).

1. Box, serving as a basis for the test capillaries, and containing all the necessary ingredients for a complete test.
2. Two glass tubes, *A* for distilled water, *B* for blood, united at one end by an u-shaped tube with a common outlet.

Tube *A*.

- a. Reipient for distilled water.
- b. Test capillary.
- c. Tube for measurement, graduated in viscosimetric units.

By a stop-cock at the end of tube *c* the water system can be separated from the pressure system.

Tube *B*.

- a. A removable receptor for blood.
- b. Test capillary.
- c. Tube for measurement (empiric scale).

3. Rubber tube, connecting the common outlet of the U-shaped tube with the glass valve.
4. Nonautomatic glass valve: enlarged glass tube with lateral hole.
5. Rubber bulb.
6. Set of small glass tubes, used as blood receptors, and several larger cleaning tubes.
7. Bottle with ammonia.
8. Bottle with distilled water.

## II. Handling the apparatus.

### A. Establishing the movements:

- a. Suction: first compression of bulb, then closure of valve hole with thumb. Release of bulb should be slow.
- b. Pressure: closure of valve hole first, then compression of the bulb.
- c. Sudden release of suction or pressure to stop the flow of the fluid columns: the thumb is lifted from the hole of the valve.

### B. Filling the glass tubes:

#### 1. Water Tube A.

- a. Cleaning tube is filled with pure distilled water.
- b. The stopcock is opened and suction produced.
- c. The cleaning tube is adjusted to opening of Tube A, so that the water meniscus touches the opening first.
- d. The water is drawn in until column reaches mark "O," where meniscus is stopped by releasing thumb from valve.
- e. Closure of stopcock.

#### 2. Blood Tube B.

- a. Blood receptor is filled with a fresh drop of blood, which must enter the tube spontaneously (any hesitation indicates that the recipient is not clean).
- b. Suction is produced first, then receptor adjusted to the opening of blood Tube B.
- c. The blood column is drawn to mark "O", and suction released.

### C. Completing the test:

- a. Menisci are controlled, that both touch mark "O".
- b. Stopcock is opened and suction produced.
- c. Stopping of suction when blood column has reached mark "1" (serum is drawn to mark "2", very thick blood to mark "0.5").
- d. Reading of position of water meniscus gives directly the relative viscosity.
- e. Both columns are forced back to mark "O", the stopcock closed and the blood expelled as quickly as possible.

### D. Cleaning the instrument:

One should clean the blood capillary immediately after use by drawing ammonia back and forth. If the apparatus is not used, pure ammonia should be left in the capillary.

The capillaries should be as clean as possible. In cases of erroneous readings there is a doubt in the cleanliness of the instrument and fuming nitric acid should be used first, then water, alcohol, and ether.

When emptying the capillaries, wet cotton should always be used to catch the liquid and air bubbles at the opening.

The blood receptors have to be cleaned at once by removing the blood with water. They are left in fuming nitric acid for twelve to twenty-four hours, after which they are rinsed with distilled water and dried on a wire gauze over a Bunsen burner.

To escape the danger of having the delicate capillaries obstructed by coagulated blood, water, sugar-solutions, serum, alcohol, etc., should be used first, until it is possible to run the test automatically.

## III. Preparing the patient:

The patient is prepared by having his hands submerged in hot water (42° C.) for five minutes, asking him to rub them together constantly. This results in a

hyperemia of the capillary net of the hand. The blood, obtained by a small incision in the end of the finger is sufficiently arterialized to give a constant value. Venous stasis increases the viscosity of the blood in consequence of an increase in the size of the red cells through the influence of carbon dioxide. Such blood shows great variation in viscosity.

#### VALUE OF THE DETERMINATION OF VISCOSITY

The viscosity is a very definite property of the blood. It is a body-constant like the temperature, and, in the same individual, remains the same, including the daily variation, over long periods. A deviation from the usual rate takes place only after drastic procedures or after a permanent change in diet. There is still a doubt whether the viscosity has a specific purpose, as, for instance, to prevent the inhibition of the tissues by the blood fluid, or whether the viscosity is the unavoidable result of certain blood ingredients. The lower limit of their concentration is indicated by the demand for supply of the body cells. The blood must carry a certain load of cellular elements and high viscous protein. The resulting viscous fluidity increases the work of the heart to many times what it would be if the blood had the viscosity of water. The viscosity in relation to heart work therefore becomes a very important factor in circulatory and cardiac disturbances. Hess believes that an increase in the number of red cells is followed by an increase in concentration of the dissolved protein. This is an additional factor of no less importance, which, however, is only mentioned in this connection; for mathematic formulation see Hess' work.

The viscosity is also a very complex property of the blood, and may be considered to represent one side of an equation, the other side of which is formed by a series of variable factors. The influence of these factors is not merely a summation, but is rapidly intensified with an increase in concentration. These factors are easily determined and the viscosity represents then a very valuable and exquisite check on the accuracy of the other values. Every deviation of the expected  $\eta$ -value demands an explanation that will lead to the discovery of an error or to a cause not yet considered. Furthermore, the viscosity represents an especially fit test for rapid controls of the blood (routine tests). Naegeli states that the determination of the viscosity must be part of an accurate blood test, but that it is of importance to have the complex value of  $\eta$  analyzed.

#### DISCUSSION OF THE VARIOUS FACTORS OF THE VISCOSITY OF THE BLOOD

Practically the most important factors which contribute to the complex value of the viscosity of the blood are the serum and the cellular elements. Naegeli enumerates some other factors, but their influence is almost negligible under the most frequently studied conditions. The influence of carbon dioxide is eliminated by the hand-bath which avoids congested blood for the determination. Nevertheless we find a specific influence of carbon dioxide in general cyanosis, which can be recognized when the precaution of inducing hyperemia is routinely applied.

Experiments which I carried out in 1919 in the physiologic laboratory of Hess have clearly demonstrated the prevalent influence of the dissolved protein in the serum. The delicacy of the method was proved by the fact that differences



of the protein were readily revealed, and that it was possible to differentiate protein by a so-called viscosimetric index. Other experiments specified the influence of the volume of red blood cells on the viscosity of the blood, at least viscosimetry was shown to give an exact determination of the cell volume (Ulmer-Bircher method).

#### VISCOSITY OF THE SERUM $\eta_1$ (PLASMA $\eta_2$ )

1. In normal conditions the viscosity of the serum ranges from 1.7 to 2.0 (plasma from 0.2 to 0.3 higher).

2. In pathologic conditions the lowest value of the serum found by Naegeli was 1.45 in pernicious anemia, and the highest 2.3 in plethora.

The influence of salt dissolved in the serum is negligible within certain limits; preponderant is the dissolved protein, and viscosimetry is therefore an approximate method of estimating the protein content of the serum.

TABLE I (Naegeli)

PROTEIN CONTENT OF THE SERUM PER CENT	VISCOSITY IN VISCOSIMETRIC UNITS
5.0	1.43
5.5	1.46
6.0	1.57
6.5	1.56
7.0	1.61
7.5	1.67
8.0	1.72
8.5	1.78
9.0	1.84
9.5	1.90

#### CELLULAR ELEMENTS

*Red Cells.*—The viscosity is directly related to the number of red blood cells or, more correctly, to the total volume of red blood cells. A practical method of estimating the exact total volume of the red blood cells and the volume of each cell is discussed in Section III.

The volume of the cells determines the viscosity of the blood. This is a very important fact for the differential diagnosis of pernicious anemia and secondary anemia. While in pernicious anemia the size of the red cells does not permit the viscosity to decrease in proportion to the low count, in secondary anemia the viscosity is very low, proving that there are no cells of increased size. This interesting fact shows, too, that the megalocytes in pernicious anemia must be much more prevalent than has been believed.

*White Cells.*—These cells do not influence the viscosity in normal blood owing to their small number, but they have a marked influence in pathologic conditions in which the definite relation of the cell volume to the viscosity permits a differential diagnosis, example of which is shown in Table II.

TABLE II (Naegeli)

	MYELOID LEUKEMIA	LYMPHATIC LEUKEMIA
Red cells	4,540,000	5,650,000
Hemoglobin	90.0 per cent	110.0 per cent
Color index	1.0	1.0
Leucocytes	396,000	44,240
Viscosity	7.3	4.1
	Viscosity is markedly increased (not due to number of red cells)	Viscosity is not increased, although the number of red cells is large

## THE DETERMINATION OF THE ALBUMIN AND GLOBULIN PERCENTAGE IN THE SERUM

In discussing the viscosity of the serum as a factor of the total value, it has been mentioned that viscosimetry furnishes approximately the percentage of protein content of the serum. Large series of experiments have shown that there are discrepancies which can only be explained by the differences in viscosity of various proteins, because for the same amount of protein different viscosimetric values have been obtained. The explanation has been furnished by the experiments of Heyder and Rohrer, who showed that the protein consists of two substances of different viscosity, albumin and globulin; the former less viscous than the latter. It is therefore to be expected that a solution of protein rich in albumin should have a lower viscosity than a solution of the same percentage of protein rich in globulin. Heyder and Rohrer have established a definite relation among the three factors: viscosity, protein percentage, and albumin-globulin mixture. They established the viscosimetric curve of albumin solutions of different concentration and one of globulin solutions under the same conditions. The viscosity was measured with the viscosimeter of Hess, and the concentration with Pulfrich's refractometer, which, according to Naegeli, permits a very accurate determination, requiring only a few drops of serum (for notes on handling the apparatus see instructions with it).

The albumin and globulin curves determine a field which includes all possible rates of any mixture of albumin and globulin. The important part of this field is reproduced in Fig. 3, by means of which it is possible to interpolate the unknown factor, the albumin-globulin mixture in percentage. Charlotte Loebner, in her study of the serum conditions in carcinoma, describes a method of obtaining a small quantity of serum. Twenty drops of blood are collected in a test tube 5 cm. long and 1.5 cm. in diameter; the tube is corked and put in an ice box for a few hours. The serum is aspirated with a thin pipette, the point of which is deflected in a right angle. If the serum is not perfectly clear, it must be centrifuged. Naegeli provides the following data:

"The serum protein in normal individuals contains 60 to 80 per cent albumin and 20 to 40 per cent globulin.

"The relation A:G (albumin to globulin) is almost constant during the day.

"No difference exists between arterial and venous blood.

"Intake of food and water does not change the relation A:G.

"Muscular activity has no influence on the albumin-globulin ratio.

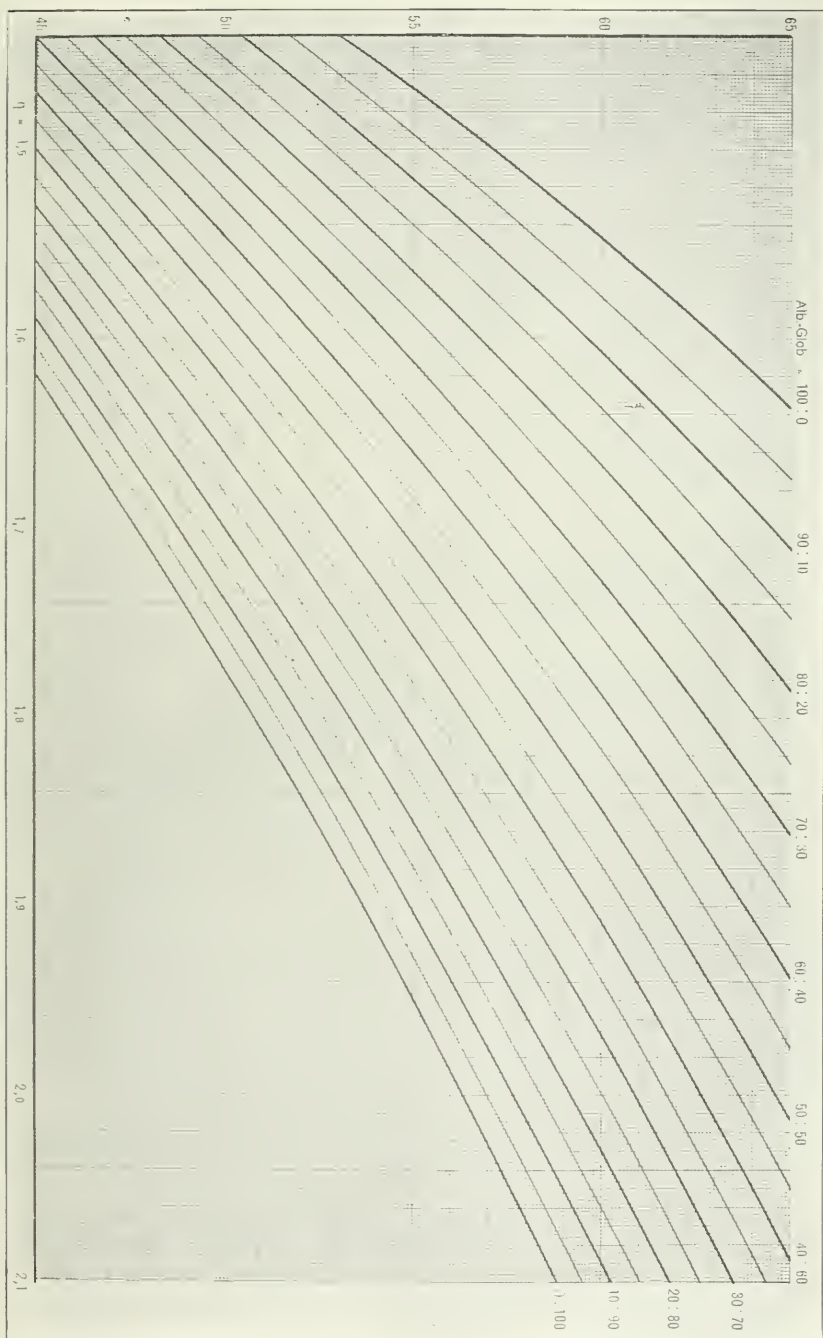


Fig. 3.—Viscosimetric and refractometric function of albumin-globulin mixtures (Chart used at the clinic of the University of Zurich). This chart allows the interpretation of an unknown mixture of albumin and globulin when the viscosity and the refraction of the serum have been determined.

"The newborn have a low protein content and low globulin percentage. There is a marked difference between the blood of the mother and the blood of the child.

"The proportion of albumin to globulin shows the following deviations in pathologic conditions:

"Hunger, diseases of the heart and lungs, and cachexia: The globulin content in venous blood is increased in comparison with the globulin content in the arterial blood.

"Chlorosis: In extreme hydremia no change in the A:G relation can be found. Although the protein content of the serum increases after treatment, the relation A:G remains unchanged. Any deviation from this rule puts the diagnosis of chlorosis in doubt or complications must be considered.

"Pernicious anemia: In progressive cases the albumin content steadily increases.

"Secondary anemia (carcinoma or tuberculosis): The progress of the disease is accompanied by a marked increase of the globulin factor."

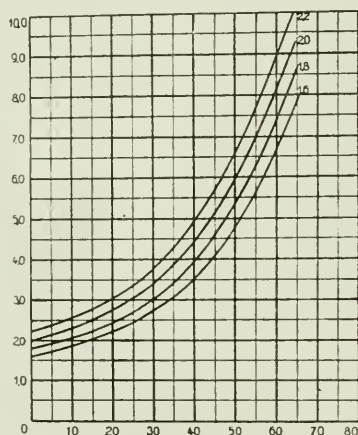


Fig. 4.—(From textbook of O. Naegeli). Viscosimetric function of the red blood cell concentration of fresh blood. The volume of red blood cells for each unit of blood can be determined by means of this chart when the viscosity of whole blood and of the suspending plasma is known.

The albumin globulin proportion of the blood serum in syphilitic patients has been studied by this method. The results will be reported soon by Bircher and MacFarland.

#### THE DETERMINATION OF THE TOTAL BLOOD CELL VOLUME AND THE EXACT VOLUME OF A SINGLE RED CELL

A hyperbolic curve was obtained in measuring the viscosity of blood samples which were so arranged that the first consisted of serum only and the following samples had a steadily increasing amount of suspended red cells. With serums of higher protein concentration similar curves were almost absolutely parallel with the first one. Figure 4 shows a series of parallel curves starting at different viscosimetric values according to the viscosity of the suspending serum.

To determine the total cell volume it is only necessary to know the vis-



cosity of the blood and the viscosity of the plasma. The viscosity of the plasma  $\eta_2$ , indicates which curve has to be chosen (point  $\eta_2$ ) and the viscosity of the whole blood  $\eta$  will be found lying on this curve. The corresponding point on the abscissa indicates the percentage of the cells. By an easy interpolation it is possible to cover every case. It is advisable to use plasma instead of serum, because the plasma constitutes the real suspending fluid for the red cells. Naegeli advises adding to the blood some Hirudin crystals immediately after withdrawal, and separating the plasma from the cells by sedimentation or centrifugalization. This method is not absolutely exact, as I have been able to show, but compared with any other clinical method the difference is not more than  $\pm 3$  per cent (Alder-Naegeli).

To get the volume of one red cell it is necessary to divide the total volume of red cells in 1 c.mm. of blood, as determined by this method, by the number of cells. A total volume of red cells of 0.44 c.mm. is found in 1 c.mm. blood containing 5,000,000 cells and therefore one red cell equals 0.44: 5,000,000, equals 88 cubic micra, or, to simplify the calculation, the volume figure of 1 c.mm. blood is multiplied with 1000 and divided by the cell number expressed in millions, for example

$$440:5 \text{ equals } 88.$$

Considering the platelets and the white cells, this figure has to be reduced to 87 cubic micra or 86 cubic micra.

TABLE III

Data on pathologic subjects (Naegeli)

Cured chlorosis.....	78 to 83 (still too low)
Secondary anemias .....	59 (lowest rate)
Hemolytic anemias .....	92
Pernicious anemias .....	165 to 120

In relation to cardiovascular diseases the hydrodynamic significance of the constitution of the blood should be considered with much more attention than it has received. Einstein and Hess have repeatedly discussed the problem and have given definite formulas for the hydrodynamic influence of the total cell volume. In an elaborate study Hess<sup>31</sup> showed the relation between the heart work and the red cells. He states that not the amount of blood thrown out by the heart but the load of cells and hemoglobin carried by the blood is the index of the effect of heart work. A greater volume of red cells for each unit of blood volume is more effective than a smaller volume, but the viscosity, being opposed to the heart work, increases accordingly and these two opposite effects reach a point of equilibrium, where viscosity counterbalances the effective load. This point has been calculated theoretically by Hess and called the optimum phase. (It lies between 40 per cent and 50 per cent red cell volume.) This same optimal condition exists in normal blood.

The knowledge of the volume percentage is as valuable for the functional examination as for diagnostic purposes. This point has been discussed by Naegeli, whose findings are given in Table IV.

TABLE V

	<i>Chlorosis</i>			
	HEMOGLOBIN PER CENT	RED CELLS MILLIONS	FORMED ELEMENTS, VOLUME PER CENT	VISCOSITY
Chlorosis	60	4.56	33.5	3.4
Chlorosis	78	4.272	30.9	3.1
Cured chlorosis	99	4.60	40.9	4.2
Cured chlorosis	97	4.58	40.7	4.0

The number of red cells is normal, but the percentage volume is decreased. The red cells are more or less collapsed because of their insufficient filling with hemoglobin. Nothing is more interesting than to watch by this method the gradual filling out of the cells. The cured patients show normal blood cells with their relative viscosity at a normal rate.

	<i>Anemia</i>			
Secondary anemia	37	2.03	17.9	2.3
Hemolytic anemia	52	2.56	23.6	2.7
Pernicious anemia	44	1.08	17.9	2.4
Pernicious anemia	96	3.57	43.1	4.3

In secondary anemia red cells, percentage of volume, and viscosity decrease at the same rate; there is no change in the size of the red cells.

In pernicious anemia the percentage of volume may be found to be the same as in secondary anemia, while the number is only one half. This is clearly indicated by the viscosity.

#### SOME VISCOSIMETRIC DATA IN RECENT LITERATURE DEMANDING FURTHER INVESTIGATION

The viscosimetric factor of the blood has been studied in the following conditions:

1. *Morbus Basedowii (exophthalmic goiter)*.—Kaess found the following variations: (a) Decreased viscosity in pure sympathicotonic cases. (b) Increased viscosity in pure vagotonic cases. (c) Inconstant variations in mixed conditions. (d) Return to normal rates about three weeks after successful operation.

2. *Pregnancy*.—Pellisier found that in (a) pregnancy with albuminuric complication a decrease in viscosity and a simultaneous increase in blood pressure indicate an obstruction of the kidneys; and that in (b) pregnancy with tuberculosis, with low arterial tension, an increase in viscosity indicates a serious form of disease.

3. *Infancy*.—Weill and Gardère believe in the prognostic value of the viscosity in severe nutritional disturbances. They found (a) decreased viscosity in anemia and eczema and (b) increased viscosity in pneumonia and congenital cardiac lesions.

4. *Surgical Diseases*.—Very similar results have been obtained by Bolognesi, Müller, Oehlecker, Simon, Süssenguth, Frischberg, and Mayesima.

A. The normal course of the viscosimetric factor in aseptic operations (Müller): (a) Postoperative increase (reaches the highest value the first day). (b) Gradual decrease in the following days. (c) Fall below the normal (phase of exhaustion). (d) Regaining the normal rate (phase of recovery).

B. Fracture of the skull (Oehlecker). The injury of the marrow of the

bones is followed by an increase of the viscosity of the blood. This is therefore an aid in the differential diagnosis of the injuries of the head.

C. Appendicitis (Simon). An increase in viscosity demands an immediate operation. In acute cases an operation should be performed regardless of a normal viscosity (Table V).

D. Differential diagnosis of rupture of extrauterine gravidity, perityphlitis, involvement of the adnexa uteri (Oehlecker, Simon, Süssenguth). (a) Low values point to involvement of the tubes or ovaries and may exclude appendicitis. (b) Extremely low values indicate internal hemorrhages, for example, after rupture. (c) High values are found in perityphlitis.

E. The question of drainage of the abdominal cavity (Oehlecker). The increased viscosity points to drainage of the abdominal cavity after operation, even if the pathologic changes do not seem to be dangerous.

F. Prognosis (Oehlecker, Simon, Süssenguth). (a) A steady decrease of the viscosity in secondary anemias denotes a poor prognosis. (b) If in peritoneal infections viscosity does not decrease after operation, the danger is not removed, or complications are developing. (c) In most of the cases of peritonitis an increase in viscosity indicates death.

5. Normal viscosimetric values as a standard for comparison with the rates obtained in acute surgical cases (Hess<sup>23</sup>).

TABLE V

AGE	VISCOSIMETRIC RATES	
	MALES	FEMALES
0 to 10 years	3.8	3.8
10 to 20 years	4.4	4.2
20 to 35 years	4.7	4.2
35 to 50 years	4.9	4.4
50 to 81 years	4.6	4.5

## SUMMARY

The problem of viscosity of the blood was taken up by Poiseuille in 1847 and, though a considerable amount of work has been done since then, the medical profession, especially in America, has paid little attention to it.

The value of determining the viscosity is threefold:

1. In circulatory questions, viscosity should be considered. Red cells and viscosity are the two opposite factors which have a determining influence on the effect of heart work.

2. The viscosimetric factor is an excellent check of the normal blood test, because it depends on the different constituents of the blood. It is a delicate indicator for changes in their relation, and on this fact are based some valuable diagnostic information.

3. The viscosity is an accurate means of analyzing the constituents of the blood: the quantity and quality of protein dissolved in plasma may be studied as well as the exact volume of the cellular elements. On these determinations are based the diagnostic applications supported by Naegeli.

A tabulation of some valuable findings is given with the intention of promoting further studies along this line.

The viscosimeter of Hess has overcome all the difficulties necessarily adherent to the determination of a dynamic factor in a living body. The test requires one drop of blood, and is easily carried out in thirty seconds, giving an accuracy of clinical value.

The viscosity of blood is compared with the viscosity of distilled water under the same conditions and is therefore relative. The normal values depend on age, sex, and constitution: the average is 4.5.

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## THE TREATMENT OF ACUTE PHOSPHORUS POISONING\*

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PHOSPHORUS poisoning is at present not so frequent, consequently not so important as formerly, yet it does occur, and the present investigation was instigated by a recent case of phosphorus poisoning successfully treated, by a method which involved the theories discussed in this paper, on the service of Dr. Charles Speneer Williamson in Cook County Hospital. Since red phosphorus is practically nontoxic, the statements which follow apply only to yellow phosphorus. Yellow phosphorus is only slightly soluble in water, absorption takes place relatively slowly, and elimination is also retarded, phosphorus having been found by Starek in the feces three and one-half days and in the vomit two days after the ingestion of a fatal dose. For these reasons the stomach tube, emetics and purges may be useful during the first day and many writers recommend them. The use of emetics seems superfluous. Blood transfusion and the oxidation of the phosphorus with potassium permanganate, hydrogen peroxide, oil of turpentine and copper sulphate have been tried (Sollmann, Hare, Kobert). The effectiveness of all these treatments has been repeatedly questioned. Old "ozonized" French oil of turpentine may be useful but rectified German and American turpentines are ineffective. Dilute copper sulphate envelopes the globules of phosphorus with a coating of oxidized copper which retards absorption. Copper sulphate also acts as an emetic if given in excess but studies by Thornton and Hare indicate that when given in doses large enough to be effective, it is as dangerous a poison as the phosphorus. This is probably due to a depression of the emetic mechanism, for a similar lack of response to cathartics is often noted in cases of paresis of the bowel in infections, etc., consequently lavage is indicated in all such cases since emetics and cathartics may be ineffective, and cannot be so efficient as lavage. When an oxidizing agent is used in phosphorus poisoning, it is usually recommended that it be followed by a saline purge such as magnesium sulphate or magnesium citrate. Sodium bicarbonate is recommended to prevent acidosis. Magnesium citrate may be useful to combat acidosis since it is the salt of an organic acid and will be oxidized to a carbonate if absorbed. It is recognized that when the systemic symptoms set in, these methods are practically useless and the treatment must be mainly symptomatic.

Warning is given by most all authors to avoid oils, fats and milk while any phosphorus remains in the alimentary tract, since they increase its solubility and rate of absorption either along with the fatty derivatives formed by the lipases of the duodenum or, due to the precipitation of the phosphorus

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on the walls of the gut during the absorption of the fatty menstium in such a finely divided condition that the large surface exposed facilitates absorption thus hastening the fatal termination of the case.

I thought that advantage might be taken of the relatively high solubility of phosphorus in oils in the treatment of acute phosphorus poisoning, especially since it extends also to the mineral oils. It was thought that castor oil might dissolve phosphorus and since castor oil is but slightly absorbed it could be used to sweep the phosphorus out of the alimentary canal before any appreciable absorption had taken place. Only a few experiments were undertaken with castor oil however because of the well-known fact that dogs are hard to purge with castor oil and because it is absorbed by dogs to a considerable extent. It is also used by human beings notably the Chinese who use it as a food fat. This, however, is not an absolute contraindication, since, if used with other cathartics, absorption would be lessened. Cottonseed oil also dissolves phosphorus quite readily and attempts were made to take advantage of this fact first and then sweep the resulting solution out of the body with a saline cathartic. These attempts were not very successful because of the apparent paresis of the bowel. The theory, however, is valid and unquestionable if any cathartic will act, and there is no reason to assume that if castor oil, which is an excellent solvent for phosphorus, be used in addition to a saline cathartic, the results would be more efficient than if saline alone were used. However we have a more reliable treatment, consequently there is little gained in pushing this theory to the limit. This treatment was found to be the use of the mineral oil, liquid petrolatum, which is not absorbed, consequently it has only a benign influence.

#### METHODS

Dogs were selected of about the same size (10 to 12 kilograms) and fed for several days before being given phosphorus which was administered in solution with oil or carbon disulphide on an empty stomach. Twenty-five dogs in all were used. The following protocols give the procedure followed:

**SERIES A.**—Two dogs were given 0.5 gram of phosphorus dissolved in cottonseed oil; two more were given the same dose of phosphorus dissolved in 50 c.c. castor oil and two more the same dose dissolved in 50 c.c. of liquid petrolatum. Defecation took place in all but the first two. The first four died in from one to three days and the two given liquid petrolatum are still living after one month without impairment of appetite or harmful symptoms except for the first three hours.

**SERIES B.**—Five dogs (1) 1 c.c. of carbon disulphide alone (2) 0.5 gram phosphorus dissolved in 1 c.c. carbon disulphide (3) the same dose as in No. 2 but followed one hour later with one ounce of magnesium sulphate, (4) the same dose as in No. 2 but followed in one hour with 50 c.c. of castor oil, (5) the same dose as No. 2 but followed in one hour with 100 c.c. of liquid petrolatum. All dogs defecated either spontaneously or under the influence of the cathartic. The control dog No. 1 and the dog given liquid petrolatum alone survived as did two additional dogs treated as No. 5 on the following day.

**SERIES C.**—Four dogs were each given 0.5 gram phosphorus dissolved in 10 c.c. of cottonseed oil and after 40 minutes two were given one ounce of magnesium sulphate and two 50 c.c. of liquid petrolatum. Defecation occurred in all four dogs. The first pair survived for two days and the second pair for four and one-half days.

In Series A and B it is evident that neither castor oil nor magnesium sulphate prolong the life of a dog receiving a fatal dose of yellow phosphorus, but that liquid petrolatum given one hour after taking phosphorus furnishes complete protection against the onset of harmful symptoms. In Series C it is evident that liquid petrolatum prolonged the life of the dogs for two days even when the phosphorus had been given dissolved in a small volume of cottonseed oil, but that the cottonseed oil had facilitated the absorption of the phosphorus to such an extent that death was delayed only.

Liquid petrolatum is physiologically inert and acts entirely by reason of its physical properties. Its use is recommended in the treatment of phosphorus poisoning. Since liquid petrolatum is a harmless, and nonirritating cathartic, it may be used to delay absorption from the gut in many, perhaps all cases, of poisoning.

In the experimental work, I have not investigated the value of lavage. This seems to me to rest on such solid ground that in addition to any other treatment it should be most relied on, and most thoroughly used. Petrolatum could be used advantageously also in the lavage.

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# THE SYNTHESIS OF ARSPHENAMINE AND A STUDY OF SOME OF ITS INTERMEDIATE DERIVATIVES\*

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## INTRODUCTION

ARSPHENAMINE is the name under which the synthetic arsenic compound, formerly known as "salvarsan" or "606," is now licensed to be manufactured in the United States.

Previous to the outbreak of the war in Europe, the entire world's supply of this drug was controlled by German manufacturers. At present, it is being made in France, England, Japan, Russia, Canada and the United States. Its production on a commercial scale was begun in this country in the latter part of 1916, and a sufficient quantity is now being turned out to meet our immediate needs. The American chemist has not only mastered the details of the process of manufacturing this material on a commercial scale, but also he has improved the quality of the product to such a degree that the present output is in most cases therapeutically equal to the original German stock. The conditions responsible for these achievements are briefly described in the paragraphs immediately following because of their importance as factors which led to the preparation of this paper.

Almost simultaneously with the beginning of hostilities in 1914, German shipments of salvarsan to this country began to fall off, and long before our entry into the conflict, they had ceased entirely. Fortunately for us, the manufacture of the drug was begun in England, France, Japan and Canada before this last stage was reached, and we were able to secure some of the material from these sources. The quantity available, however, was limited to that produced in excess of the home needs of these countries and their allies and small quantities produced in this country; this was far too small to supply our needs. This shortage became acute when we entered the conflict and the mobilization of our forces was begun. It then became necessary to supply the demands of our Army and Navy at the expense of the civilian population or to undertake the manufacture of the drug on a large scale.

The problem of devising a process of manufacture suited to conditions in this country was not unattended with difficulties as our chemists had little or no previous experience in the preparation of arsenic compounds. To be sure, there was a great amount of information on the subject in the chemical literature and the patent specifications were available. In fact, the entire

\*This paper was prepared in 1918 and was intended to furnish impartial information to the large numbers of consumers who were seeking details to assist them in the clinical use of the arspenamine. Paper shortage and delays postponed the early publication of the article.

The author wishes to give due credit to Dr. G. C. Lake for biological data and to Dr. A. G. DuMez for chemical data used in this article.

process of manufacture is described in the various numbers of the "Berichte der deutschen chemischen Gesellschaft." Production was further delayed by difficulties experienced in securing the necessary equipment for manufacturing on a commercial scale, and by the necessity for changes in the process brought about by the prohibitive cost of certain essential chemicals. Nevertheless, such rapid progress was made that on November 30, 1917, the Federal Trade Commission issued orders for licences to manufacture the drug to no less than three manufacturers, namely, the H. A. Metz Laboratories, Inc., New York, the Dermatological Research Laboratories, Philadelphia, and the Takamine Laboratory, Inc., New York.

As already mentioned in the beginning of this paper, the drug received in this country before the war was produced solely in Germany, where it was manufactured under the direct supervision of its discoverer, Ehrlich. It was carefully tested, both chemically and physiologically, to establish its identity and to determine its degree of toxicity. Naturally, a fairly uniform product was obtained. With the advent on the market in this country of the various brands produced by the newly established factories in the countries previously mentioned and the assurance that American manufacturers would soon be producing, it became necessary to establish standards for the drug in order to insure a product of good quality and to guard against the appearance of spurious material. This the United States Public Health Service undertook to do, and on November 22, 1917, tentative rules and regulations for the manufacture and sale of the drug were promulgated. (See Public Health Reports, 1917, v. 32, No. 49, p. 2071).

In order that the Public Health Service might keep in as close touch as possible with developments in the process of manufacture and be of the greatest assistance in aiding the manufacturers to improve the quality of their respective products, it was highly desirable that a representative be stationed at one of the manufacturing plants. The fulfillment of this desire was made possible through the courtesy of H. A. Metz of the H. A. Metz Laboratories, Inc., New York, who offered the Public Health Service the use of his plant gratuitously. As a result, Dr. C. N. Myers was detailed to this plant to make a study of production on a commercial scale and to supply the Hygienic Laboratory of the United States Public Health Service with the materials, including intermediates, by-products, et cetera, necessary for carrying out such pharmacological and toxicological studies as might have a bearing in this connection.

This paper deals with some of the results of these studies insofar as they pertain to the process of manufacture and the toxicity of a few of the compounds isolated or prepared by the author.

#### MANUFACTURE OF ARSPHENAMINE

The processes for the manufacture of arspenamine herein described are intended to apply to the preparation of a product identical with that originally made in Germany under the supervision of Ehrlich. This statement is made because of the fact that the physical and chemical properties of some of the

products now on the market indicate that they differ from the original in their make-up.

The mother substance, or material which yields arspenamine, when reduced under the proper conditions, is 3-nitro-4-hydroxyphenylarsinic acid, commonly known in the industry as "nitro oxy." There are three well-defined methods of preparing this compound in use. Descriptions of these methods follow.

*Method 1.*—The following is perhaps the most convenient method for preparing "nitro oxy" because of the ready accessibility of the materials in normal times.

Mix 25 g. of arsenous chloride with 15 g. of dimethylaniline and, after the first heat of reaction is over, heat at the temperature of a water-bath for two hours. Pour the syrupy liquid into ice-cold water and treat with an excess of an aqueous solution of caustic soda until the p-dimethylaminophenylarsenous oxide is dissolved. The small amount of hexamethyltriaminotriphenyl arsine formed is filtered off and the unaltered dimethylaniline removed with any suitable immiscible solvent, such as petroleum ether. The filtrate is then treated with 30 per cent hydrogen peroxide, and the p-dimethylaminophenylarsinic acid precipitated by means of acetic acid (Michaelis, 1908).

This arsinic acid is sparingly soluble in cold water or alcohol, dissolves readily in hot alcohol, hot dilute acetic acid, mineral acids or alkalis. It crystallizes in needles from hot water and hot alcohol, and sublimes without melting. It forms a sodium salt (N-dimethyl-atoxyl) which corresponds to the formula  $(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{AsO}(\text{OH})\text{ONa} \cdot 5\text{H}_2\text{O}$ .

Suspend 23.5 g. of the finely powdered p-dimethylaminophenylarsinic acid in 300 c.c. of glacial acetic acid and effect solution by the addition, at ordinary temperature, of 9.8 g. of 62 per cent nitric acid. Upon the addition of 20 c.c. of acetic anhydride, the solution becomes yellow and deposits a heavy precipitate consisting of pure 3-nitro-4-dimethylaminophenylarsinic acid. By warming the latter with 2.5 parts of 40 per cent aqueous solution of caustic soda at 85° C. for 3-4 hours, the corresponding hydroxy compound, "3-nitro-4-hydroxyphenylarsinic acid or nitro oxy," is obtained.

Contamination of the "nitro oxy" with the nitro-dimethylamino compound mentioned above should be avoided as the latter yields 4:4'-tetramethyl-3:4:3'-4'-tetraminoarsenobenzene in the final stages of reduction with alkaline hydrosulphite in the presence of magnesium chloride. Upon subsequent treatment with methyl muriate, the tetrahydrochloride is formed (Karrer, 1913).

An alternative and preferable method of nitrating the dimethylaminoarsinic acid consists in dissolving 100 g. of the material in 250 g. of 60 per cent sulphuric acid and slowly adding a mixture of nitric acid (sp. gr. 1.49), 35 g. and sulphuric acid (60 per cent), 150 g., allowing the temperature to rise to 30° C. This temperature is maintained for half an hour or longer and the mixture poured on broken ice. Dissolve the yellow precipitate in aqueous sodium carbonate, filter, and precipitate the nitro-compound by the addition of a very dilute mineral acid. After purification by crystallization from hot water, the compound is hydrolyzed as described above yielding "nitro oxy."

The foregoing nitration may be carried on in concentrated sulphuric acid by keeping the temperature below  $10^{\circ}$  C.

If the nitration is carried out in more dilute solutions, or if sodium nitrate in sulphuric acid (1 to 4) is added to p-dimethylaminophenylarsinic acid dissolved in sulphuric acid (1 to 4) and heated to  $90^{\circ}$  C., one obtains 4-methyl-nitrosoaminophenylarsinic acid. On the other hand, a dinitro-compound will be obtained if there is an excess of nitric acid used and the dilution of the solvent is about 1 to 4. This process yields chromo isomerides in which there is an

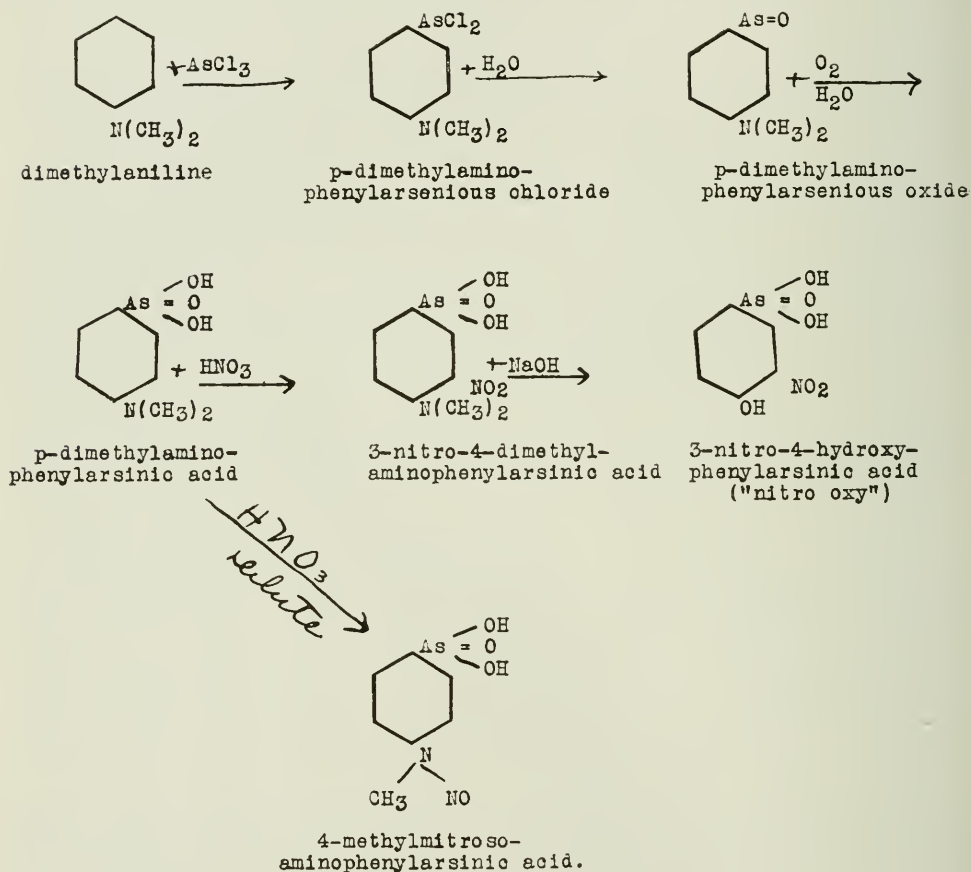


Fig. 1.

orientation of the substituent groups or in which the nitro groups occupy different positions in the aromatic nucleus. One isomer dissolves in aqueous sodium carbonate, hot water, alcohol, or acetone, separates in rosettes of bright yellow, prismatic crystals, and melts with decomposition at  $161^{\circ}$  C. The other yields small, red, four-sided plates, less soluble in hot water than the yellow modification and melts with decomposition at  $158^{\circ}$  C.

Advocates of the above method of preparing "nitro oxy" claim a very smooth nitration of the p-dimethylaminophenylarsinic acid leading to a series of definite compounds which are not always obtained by the circuitous routes



employed in the other methods. Further, this method has additional advantages in that (1) p-dimethylaminophenylarsinic acid is readily prepared from easily accessible reagents, dimethyl aniline and arsenous chloride; (2) the dimethylamino complex is removable quantitatively by hydrolysis, the products being O-nitrophenylarsinic acid (3-nitro-4-hydroxyphenylarsinic acid or "nitro oxy").

The structural formulas in Fig. 1 represent the general changes involved in the formation of "nitro oxy" by this method.

*Method 2.*—Another procedure which may be followed in making "nitro oxy" consists in stirring together 94 parts of phenol and 151 parts of crystallized arsenic acid and heating this mixture at 150° C. for four hours. The mass, which becomes dark colored, is extracted with warm water when cool and the filtered solution is concentrated *in vacuo*. The concentrate is extracted repeatedly with acetone, and the solvent is finally evaporated leaving crude phenol-p-arsinic acid as an oily liquid which gradually solidifies. The acid is purified by crystallization from glacial acetic acid, water, alcohol, acetone or dilute mineral acids. It readily forms salts with alkalis, and purification may be effected by the formation of an alkaline salt and subsequent treatment with a concentrated mineral acid (Farbwerke vorm Meister Lucius and Brüning, 1909).

This acid may also be prepared by the Bart process in which p-aminophenol is diazotized in hydrochloric acid and warmed with alkaline sodium arsenite. The solution is neutralized, filtered, boiled with animal charcoal, and concentrated, yielding sodium phenol-p-arsinate. The acid is precipitated from the salt by a mineral acid, preferably hydrochloric acid (Bart, 1912).

A third method by which the acid may be obtained consists in diazotizing a hydrochloric acid solution of p-arsanilic acid, evaporating to dryness and extracting the free acid formed with acetone (Barrowcliff, Pyman and Remfry, 1908).

To prepare "nitro oxy" from the above acid, a mixture of 39 c.c. of nitric acid (sp. gr. 1.4) and 39 c.c. of concentrated sulphuric acid is added slowly to a solution of 144 g. of the sodium salt dried at 80° C. and dissolved in 450 c.c. of concentrated sulphuric acid at 0° C. The temperature is kept between 0° and 5° C. The temperature after the addition of the nitric acid is not allowed to rise above 10° C. The acid solution is finally poured into 2500 c.c. of cold water and the precipitated "nitro oxy" collected after 24 to 48 hours (Benda, 1911). Careful work is necessary in this procedure as too energetic nitration leads to the formation of the dinitro-compound (3:5-dinitro-4-hydroxyphenylarsinic acid) which may be detected by the red color obtained with alkali and sodium hydrosulphite. High temperatures are to be avoided for the same reason. The yield of "nitro oxy" by this procedure never amounts to more than 75 per cent of the theory.

Because of the fact that the dinitro-compound was prepared for experimental purposes, a description of the method by which it was actually made is given at this point. Twenty grams of dry phenol-p-arsinic acid were added in small portions to 60 c.c. of concentrated sulphuric acid cooled to 0° C. by using a

freezing mixture. To this mixture 13.4 c.c. of nitric acid (sp. gr. 1.52) were added drop by drop with constant stirring, keeping the temperature between 15° C. and 20° C. The stirring was continued for two hours at ordinary temperature. The mixture was then poured on 300 grams of ice and by scratching the container and cooling to 10° C., the compound separated out as lustrous, pale yellow leaflets (Benda, 1912).

The dinitro-product may also be obtained from 3:5-dinitro-p-arsanilic acid. The acid is heated with 10 per cent caustic potash at 90° C. until the ammonia is eliminated, when the dinitro-compound is precipitated by the addition of hydrochloric acid.

The following structural formulas (Fig. 2) explain the important chemical changes which occur in the preparation of "nitro oxy" from phenol.

*Method 3.*—A third method for the preparation of "nitro oxy" is based

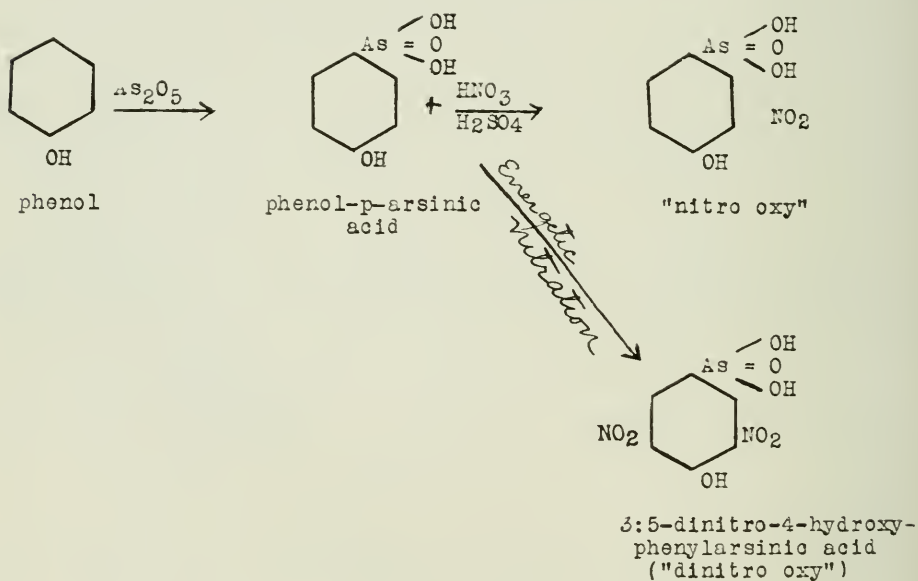


Fig. 2.

on the use of aniline as the material for beginning the synthesis. The aniline may be arseniated by either of the following procedures.

The direct combination of arsenic and aniline is effected by heating in a suitable container crude arsenic acid (containing 75 per cent or more  $\text{As}_2\text{O}_5$ ) until it becomes practically 100 per cent  $\text{As}_2\text{O}_5$ . Aniline (186 grams) is cooled to about 0° C. and 140 grams of the dry arsenic acid is slowly added with constant stirring. The mixture soon thickens and finally a crystalline mass forms. At this stage the stirring should be vigorous enough to pulverize the material. The composition of this product is probably an arsenate of aniline. The temperature is slowly raised to 170° C. with continuous stirring, allowing the powder to melt slowly. The apparatus in which this operation is carried out should be equipped with an oil bath and a condenser to dispose of the aniline which is volatilized. Finally the temperature is raised to 190° and main-

tained with continuous stirring for two hours. The product is then mixed with water, rendered alkaline, and the excess of the base distilled off in steam. The residue is allowed to cool, filtered, concentrated, and neutralized with hydrochloric acid, when the crude arsanilic acid separates out. This product is dissolved in aqueous caustic soda to a faintly alkaline reaction. At this stage, the material is sometimes boiled with animal charcoal (usually the free acid is boiled with charcoal as there is less danger of splitting off the arsenic) and filtered into ethyl alcohol, when the Sodium Salt (atoxyl) separates out. The impurity, which is secondary arsanilic acid or di-4-amino-diphenylarsinic acid, remains in solution. The free arsanilic acid is liberated from atoxyl by means of dilute hydrochloric acid (Adler, 1908). This procedure for combining arsenic and aniline is known as Béchamp's condensation (Béchamp, 1863). It is sometimes spoken of as the melt method.

Para arsanilic acid is sparingly soluble in ethyl alcohol, water, more so in methyl alcohol, insoluble in ether and the other organic solvents. It is amphoteric, dissolving in excess of mineral acid, and being reprecipitated by sodium acetate. It is partially hydrolyzed by boiling with water. The product in its purest condition is a white crystalline solid. Occasionally it has a slight pink to violet color.

Secondary arsanilic acid is a colorless, needle-like solid having a melting point of  $248-249^{\circ}$  ( $232^{\circ}$ ). It is sparingly soluble in water or the ordinary organic solvents, moderately soluble in glacial acetic acid, readily soluble in ethyl alcohol, dilute mineral acids in excess, and in dilute alkalis. Methods for the preparations of this compound are described by Pyman and Reynolds (1908) and Benda (1908). (Fig. 3.)

A second method of making arsanilic acid from aniline consists in preparing acetanilide and subsequently the para nitro-compound. The latter is reduced to acetphenylenediamine, arseniated by means of arsenous acid, and diazotized with sodium nitrite yielding the corresponding acetyl derivative of arsanilic acid. The acetyl group is finally removed by hydrolysis leaving the crude arsanilic acid. The product thus obtained is impure, being accompanied by tarry residues and diazo derivatives. The removal of these impurities is absolutely necessary to insure success in subsequent operations. This is accomplished as described above.

The para arsanilic acid obtained by either of the above methods may be converted into "nitro oxy" by direct nitration of hydroxyphenylarsinic acid, which is first prepared, or by nitration of the oxalyl compound. The latter procedure is preferable as it yields only mononitro-compounds, whereas some of the dinitro-compound is usually formed when direct nitration is resorted to.

In the first instance, the arsanilic acid is dissolved in concentrated sulphuric acid and diazotized with the molecular equivalent (1 molecule) of sodium nitrite, the diazo solution filtered and the filtrate heated to about  $70^{\circ}$  C. The hot acid solution is then treated with barium carbonate, filtered and a small amount of sodium sulphate and animal charcoal added. The solution is then concentrated to effect crystallization. The compound obtained is the mono-sodium salt of phenol-p-arsinic acid (Bertheim, 1908, p. 1853). The free

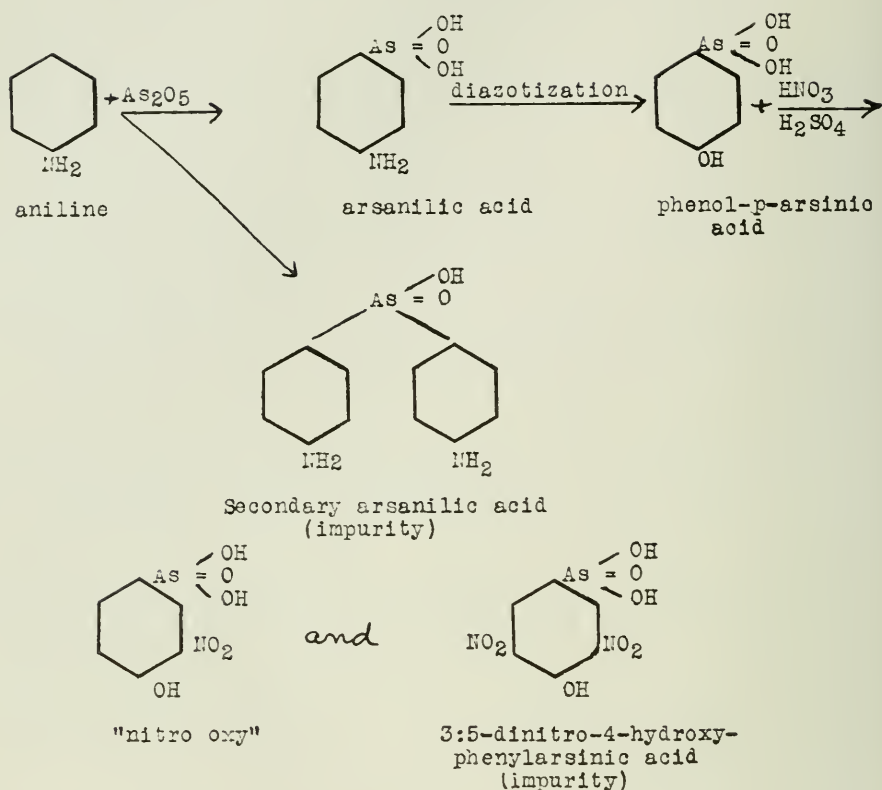
Aniline Method No. 1.

Fig. 3.

acid is liberated by means of a mineral acid. The latter may be obtained directly by carrying out diazotization in hydrochloric acid as described under method 2. Direct nitration of the diazotized compound by means of a mixture of nitric and sulphuric acids yields "nitro oxy."

When nitration is effected through the oxalyl compound the procedure is as follows: Thoroughly mix 347 g. of crystallized sodium p-arsanilate with 378 g. of crystallized oxalic acid, and heat at a temperature of 120° to 130° C. until the greater part of the water is eliminated. Then raise the temperature to 160° C. and continue to heat until the pasty mass becomes pulverulent. Mix the crude product obtained with 3000 c.c. of water and 350 c.c. of hydrochloric acid (sp. gr. 1.12), filter, and dissolve the precipitate in a mixture of 700 c.c. of cold water and 200 c.c. of 10 N caustic soda. The filtered solution, when acidified with 390 c.c. of hydrochloric acid (sp. gr. 1.12), yields oxalyl-p-arsanilic acid in the form of a fine white precipitate (Bertheim, 1911). The latter is nitrated by dissolving 116 g. in 300 c.c. of concentrated sulphuric acid, keeping the temperature down to 15 to 20° C. and adding drop by drop a mixture consisting of 26 c.c. of concentrated nitric acid (sp. gr. 1.4) and 26 c.c. of concentrated sulphuric acid. After nitration has been completed, the mixture is stirred for  $\frac{1}{2}$  hour longer, 1500 c.c. of water are added, and the



Aniline Method No. 2.

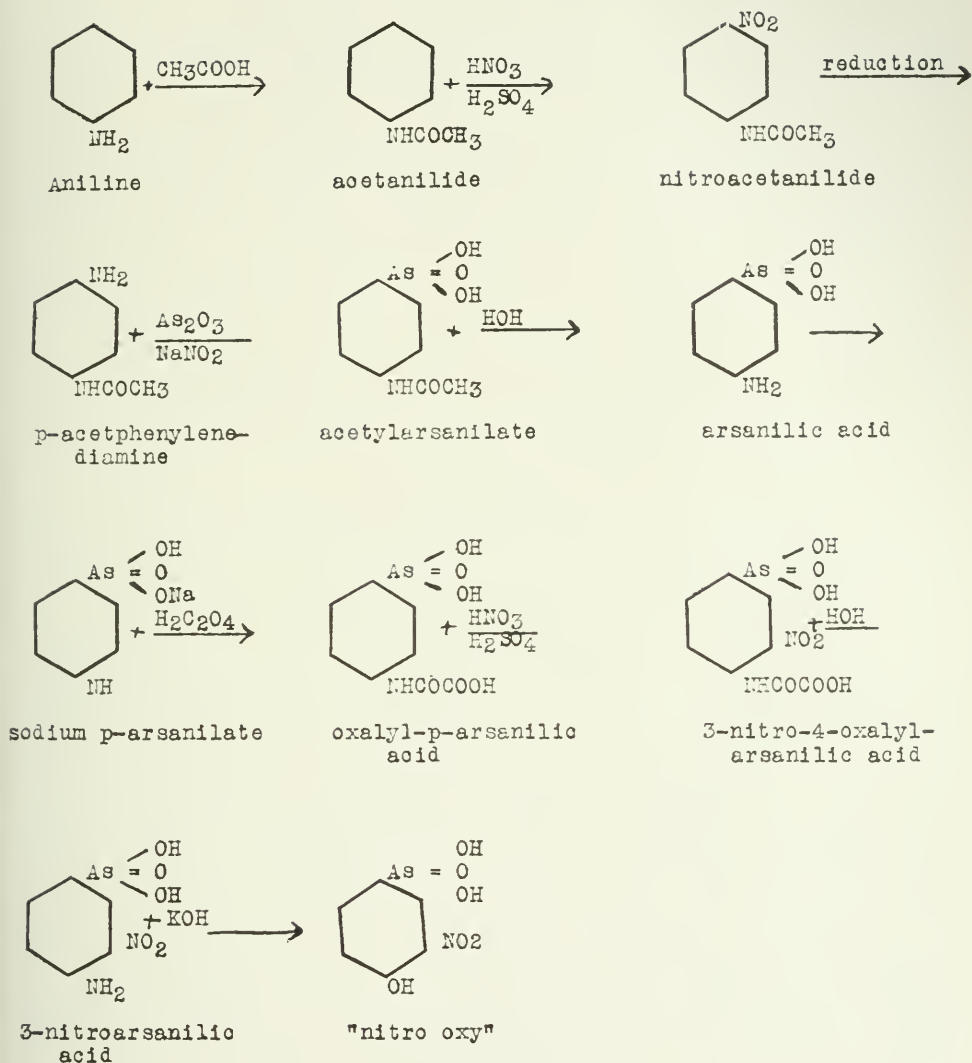


Fig. 4.

whole heated to boiling under a reflux condenser for 1 hour. On cooling, 3-nitro-4-aminophenylarsinic acid separates out in the form of long yellow silky needles (Berthelm, 1911). To obtain "nitro oxy," dissolve 500 g. of the nitro-compound in aqueous potassium hydroxide (500 g. KOH in 1500 c.c.  $H_2O$ ), and heat the solution at  $80^\circ$  to  $90^\circ$  C. until the mixture becomes nearly solid. Ice-cold water (2000 c.c.) and hydrochloric acid are added successively, the precipitate dissolved in hot water, and the filtered solution treated with sodium acetate (1 mol.) and animal charcoal. After this treatment, the mixture is again filtered and the filtrate made acid with hydrochloric acid when "nitro oxy" separates either in yellow rhombohedral plates or in tufts of almost colorless needles.

The formulas in Fig. 4 show the course of the reactions described above:

"Nitro oxy" is dimorphous in character, crystallizing in the form of yellow rhombohedral plates or tufts of almost colorless needles. It is also probable that the compound exhibits a chromoisomerism, in which there is a labile and a stable form. The work of A. Hantzsch (1910) with analogous compounds is suggestive in this connection. The substance is only slightly soluble in cold water or mineral acids, but is quite soluble in hot water forming a yellow colored solution. The color disappears almost completely on the addition of mineral acids. It dissolves readily in methyl and ethyl alcohol, glacial and 50 per cent acetic acid, but is insoluble in ether and acetic ester. It unites with alkalies forming salts which are soluble in water. The mono-sodium salt is bright yellow in color and gives an acid reaction; the di-sodium salt also gives an acid reaction but is orange colored; while the tri-sodium salt gives an alkaline reaction and occurs as either orange or red colored crystals. "Nitro oxy" readily breaks down in alkaline solution yielding o-nitrophenol and oxides of arsenic.

The "nitro oxy" used in the preparation of arsphenamine should be the product described by Berthelm. The arsenic should be in position one and the nitro-group in position three in the benzene ring. Any other arrangement of these groups, as in the other isomers, will yield a final product which will not be identical with the original salvarsan. Furthermore, the "nitro oxy" employed should be a *pure* product as the presence of impurities is one of the great sources of trouble in attempting to obtain an end product of good quality. Impurities, if present, interfere with crystallization and adhere to all products even to the final stages of the process. It has been argued by some that the removal of these impurities is unnecessary at this time inasmuch as still others are formed in the process of reducing the "nitro oxy." Experience, however, has shown that it is advisable to remove as many impurities as possible before the process of reduction is begun.

*Preparation of Arsphenamine Base.*—"Nitro oxy" is reduced by a number of reagents, e.g., sodium amalgam, sulphurous acid with hydriodic acid as a catalyst, phenylhydrazine, thionyl chloride, phosphorous trichloride, stannous chloride, certain ferrous compounds, phosphorous acid, hypophosphorous acid, sodium hydrosulphite, et cetera. Phosphorous and hypophosphorous acids attack only the arsenical group, while sodium hydrosulphite reduces both the nitro and arsenical groups. In the production of arsphenamine base, sodium hydrosulphite and certain combinations of the other reagents mentioned have been employed.

Two methods for the reduction of "nitro oxy" to arsphenamine base on a commercial scale are in use. They are known as the progressive and direct methods, respectively. When the first procedure is followed, "nitro oxy" is reduced progressively to 3-amino-4-hydroxyphenylarsinic acid, 3-amino-4-hydroxyphenyl-arsenous oxide and arsphenamine base or a similar series of compounds, whereas complete reduction is effected in one operation by the direct method. In reality, however, both procedures are continuous since the products of reduction obtained by the first method are not isolated at the

different stages enumerated. Reduction by the progressive method may be accomplished as follows:

Sodium amalgam (840 g. of 4 per cent Na) is added to 31.6 g. of "nitro oxy" in 600 c.c. of methyl alcohol at a temperature of 60° to 70° C. About 5% of the alcohol are distilled off, the residue extracted with 120 c.c. of water, the supernatant liquid decanted to remove the mercury, and the solution acidified with 150 c.c. of hydrochloric acid (sp. gr. 1.19). After 12 hours, the liquid is filtered, the filtrate boiled with animal charcoal and the clear solution treated with 52 c.c. of 10 N caustic soda. This completes the first stage, and the product obtained is crystalline 3-amino-4-hydroxyphenylarsinic acid (Ehrlich and Berthelm, 1912).

The second stage in the process consists in reducing the arsinic acid group of 3-amino-4-hydroxyphenylarsinic acid to arsenous oxide. This may be accomplished by saturating with sulphur dioxide a solution prepared by dissolving 230 g. of the arsinic acid, obtained as described above, in 2000 c.c. of water and 1000 c.c. of 2 N sulphuric acid to which has been added 50 g. of potassium iodide dissolved in 50 c.c. of water (Ehrlich and Berthelm, 1912).

The third stage, or reduction to arspenamine base, may be conducted in the following manner. Sodium amalgam (28.8 g. of 4 per cent Na) is added to 30 c.c. of water and 32 c.c. of 2 N acetic acid containing 4.98 g. of the 3-amino-4-hydroxyphenylarsenous oxide. When the amalgam is used up, a further addition is made of 25 c.c. of 2 N acetic acid and 28.8 g. of sodium amalgam. This treatment is again repeated, when reduction is usually complete as a test with sodium hydrosulphite will show. The yellow precipitate formed is arspenamine base.

Direct reduction of "nitro oxy" to arspenamine base is effected by means of sodium hydrosulphite, or sodium hydrosulphite in combination with magnesium chloride. The presence of magnesium chloride is thought by some to prevent overreduction and to assist in the removal of sulphur compounds. Our own experience, however, has shown that equally good arspenamine can be obtained with the use of sodium hydrosulphite alone. Inasmuch as sodium hydrosulphite is a comparatively little known reagent, a description of the compound is given at this point.

Sodium hydrosulphite, or "hydrosulphite" as it is commonly spoken of in the industry, is said to correspond in composition to the formula  $\text{Na}_2\text{S}_2\text{O}_4$ . It is a white or grayish-white solid containing about 2 per cent of zinc in addition to other impurities. It dissolves in water with the evolution of  $\text{SO}_2$ . It is evident that a part of its value as a reducing agent is due to this latter property, but its chief value in connection with the reduction of "nitro oxy" is claimed to be due to a change in the valency of the sulphur under the conditions of the reaction. Before a reduction can be successfully carried out with this compound, its reducing power must first be determined. This is usually accomplished by titration with an indigo solution.

The preparation of arspenamine base, using "hydrosulphite" alone as the reducing agent, is conducted as follows: About 197 g. of "nitro oxy,"

accurately weighed, are dissolved in 4500 c.c. of water warmed to 55° C. to which sufficient caustic soda to form the disodium salt has been added. If the "nitro oxy" has been properly prepared, a clear yellow solution will result. Approximately 13 liters of water and ice are placed in a porcelain or glass lined vessel of at least 30 liters capacity and a weighed amount (usually about 2950 g.) of "hydrosulphite," calculated on the reducing power, is slowly added. If the resulting solution is dark in color, it should be discarded as it will not yield a satisfactory reduction product. The solution of "nitro oxy" is slowly poured into the solution of "hydrosulphite" with constant stirring. When this operation has been completed, the temperature of the mixture will be between 10° and 14° C. At this point, the temperature is raised about 2° per minute up to 30° C., and then less rapidly until 68° C. is reached, the stirring being continued throughout the operation. When a temperature of 33° to 35° C. is reached, the arspenamine base begins to precipitate and continues to come down until reduction is completed. This usually requires that heating and stirring be continued for 1½ to 2 hours after the temperature has been brought up to 68° C. The stage at which reduction is complete is determined by removing a sample of the mixture, filtering and warming. Reduction is complete when the filtrate remains clear. When this point is reached, heating is discontinued and the precipitate allowed to settle during 1 hour. The supernatant liquid is then siphoned off and the yellow precipitate is collected on force filters of suitable size. Up to this stage, the precipitated base has been protected from the oxidizing action of the air by the sulphur dioxide liberated in the process of reduction. This protection is now continued by working in an atmosphere of carbon dioxide or other inert gas. The precipitate on the filter is sucked nearly dry and then washed with distilled water until the washings no longer give an acid reaction. It is then again sucked nearly dry when it is ready for conversion into the hydrochloride (Ehrlich and Berthelm, 1912, and others).

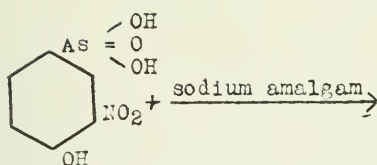
The preparation of arspenamine base by this method requires careful supervision by an experienced worker. Slight variations in temperature and the masses of the reacting substances may give rise to the formation of by-products which are considered by some as possible causes for undue toxicity. These by-products may be either sulphur derivatives or compounds of the arsine or arsenoxide type.

The free base is a yellow powder soluble in dilute hydrochloric acid and aqueous caustic alkalies, slightly soluble in alcohol, and insoluble in water and ether. It is precipitated from alkaline solutions by acetic acid.

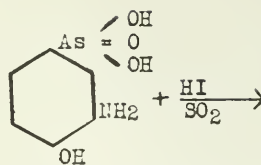
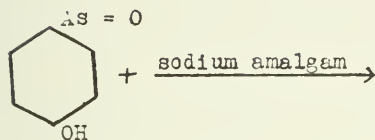
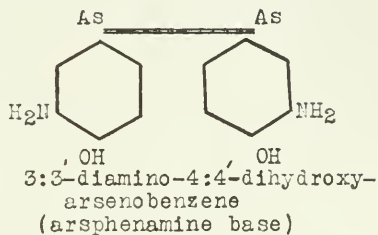
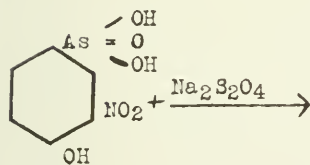
The formulas (Fig. 5) explain the reaction involved in these methods of reduction.

*Preparation of the Dihydrochloride from Arspenamine Base.*—The free base obtained as described above is dried to the proper degree, mixed with 1700 c.c. of methyl alcohol, and the calculated quantity of methyl-alcoholic hydrochloric acid (0.75 mol. HCl) is added. The solution is filtered and the dihydrochloride precipitated by pouring the solution into absolute ether with

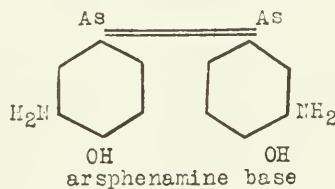


Progressive Reduction.

"nitro oxy"

3-amino-4-hydroxy-  
phenylarsinic acid  
("amino oxy")3-amino-4-hydroxy-  
phenylarsenous oxide3:3'-diamino-4:4'-dihydroxy-  
arsenobenzene  
(arsphenamine base)Direct Reduction.

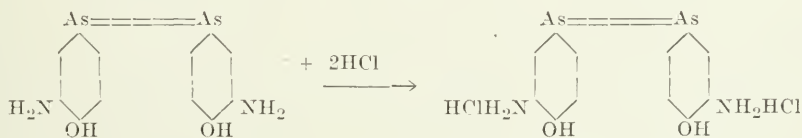
"nitro oxy"



arsphenamine base

Fig. 5.

vigorous stirring. The precipitate is collected on a force filter, thoroughly washed with ether and removed to vacuum chambers, where it is kept for 4 or 5 days before it is put into ampoules. The product thus obtained is arspenamine, or 3:3'-diamino-4:4'-dihydroxyarsenobenzene containing an amount of volatile equivalent to approximately  $2\text{H}_2\text{O}$ .



In carrying out the above operation, trouble is sometimes caused by the precipitated dihydrochloride sticking to the sides of the vessel. This is due, either to the fact that the base was not dried to a sufficient degree, or to the

presence of impurities resulting from the improper reduction of the "nitro oxy." Another source of trouble is the formation of small cracks in the material when on the filter. A partial oxidation of the material may result from the sucking of air through these fissures.

Arsphenamine is a yellow powder permanent when dry and preserved in containers with an inert gas. It is readily soluble in cold water, methyl alcohol, glycerol, ethylene glycol and dilute hydrochloric acid, slightly soluble in ethyl alcohol. The aqueous solution is acid to litmus and turns Congo-red to violet-blue. Acid aqueous solutions decompose slowly on exposure to air, changing from yellow to red or brown in color. Alkaline solutions decompose comparatively rapidly under similar conditions becoming dark brown in color. Decomposition under these conditions probably results in oxidation with the complete breakdown of the molecule as shown by the formulas in Fig. 6.

The free base is precipitated from aqueous solutions of arsphenamine by alkali hydroxides and carbonates. In the case of alkali hydroxides, it is first

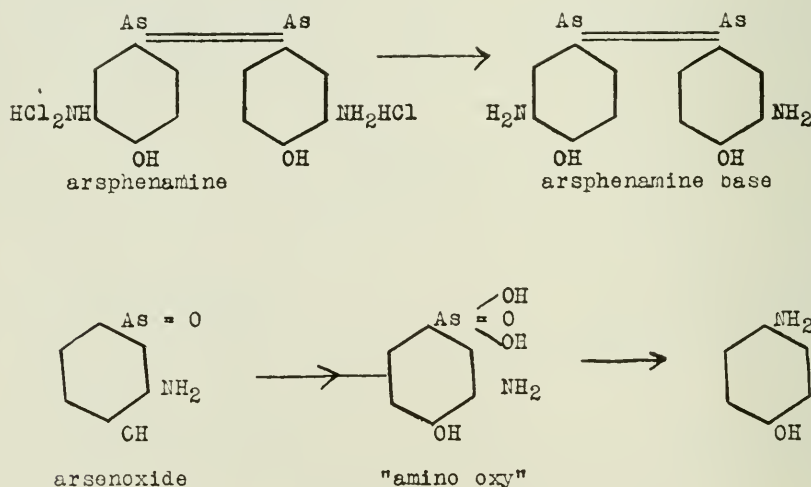


Fig. 6.

precipitated and then redissolved with the formation of the mono- or disodium salts, depending on the quantity of alkali added. Carbon dioxide precipitates the free base from solutions of the latter. For further descriptions of the properties of this compound, see Myers and DuMez (1918).

*Packing.*—The dry cakes of arsphenamine are carefully powdered in large mortars and the pulverized material passed through a fine-meshed sieve. The sifted powder is then returned to the vacuum chambers to allow any ether which may have been occluded in the caked material to evaporate. It is then divided into small portions, weighed quickly and the correct amount poured into each ampoule. The filled ampoules are placed in a vacuum chamber and the air exhausted, after which carbon dioxide or some other inert gas is allowed to flow in until atmospheric pressure is again reached. After this

operation has been completed, the ampoules are immediately sealed and labeled, the lot number being placed on each ampoule.

Due to atmospheric conditions at the time of filling, the inner surface of the ampoules may appear slightly cloudy. This condition, however, is no indication that the contents are not of good quality or have deteriorated. The factor which influences the quality of the product to the greatest extent at this stage is the care which is taken to prevent undue exposure to the air.

There is no relation between the color of the product and its toxicity. The same applies to the relation between solubility and toxicity. Products which are in true solution show no relation to toxicity, i.e., the ease of solubility is no criterion as to the toxic effects.

*(To be concluded in January issue.)*

# LABORATORY METHODS

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## A NOTE ON THE ESTIMATION OF BLOOD CHLORIDES IN TUNGSTIC ACID FILTRATES

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By JOHN B. RIEGER, M.D., DETROIT, MICH.

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IN OCTOBER, 1920, it was stated by the present author that the Volhard titration of chloride could be directly applied to the tungstic acid filtrate obtained in the Folin-Wu system of blood analysis, provided the reagents be free of chloride. The method presented<sup>1</sup> has lately been criticized on theoretic grounds by Whitehorn<sup>2</sup> who proposes that the excess of silver nitrate be titrated without removal of the silver chloride precipitate, as in the older method.

As a matter of fact neither centrifugalization nor filtration of the precipitated chloride need result in a loss of silver. The purine derivatives are never precipitated under the conditions prescribed and silver tungstate only begins to settle out when the concentration of sodium tungstate in the filtrate reaches 50 mg. per 100 c.c. Filter papers that contain chloride must, of course, not be used. The Whatman No. 2 paper has produced positive errors as high as 4 per cent, while Nos. 40 and 44, respectively, have been found free of chloride. Check analyses on mixtures of pure sodium chloride and sodium tungstate, the latter in concentrations of 25 mg. per 100 c.c., have given results invariably within 1 per cent of the theoretic figure, using either filtration or centrifugalization to remove the silver chloride.

The solubility of tungstic acid in 50 per cent nitric acid is between 15-20 mg. per 100 c.c., and is not appreciably less in the 66 per cent acid used by Whitehorn in his chloride test. Failure to obtain a turbidity with an equal volume of concentrated nitric acid is therefore entirely safe as a test for harmful amounts of tungstic acid in the filtrate. A positive test usually means incomplete precipitation of proteins and this interferes with the subsequent titration of the excess silver.

It is true that sodium tungstate can now be obtained free of chloride, but it is still advisable to purify it to get rid of the excess of alkali. In this way an accurate 10 per cent solution may be prepared and the juggling with acid to secure a complete precipitation of protein is obviated. Purification by alcohol precipitation as recommended by Folin is no doubt the better way, but pure alcohol cannot readily be obtained by workers outside of governmental or educational institutions and its recovery by distillation is prohibited, so that many are compelled to use an alternative procedure, such as described by the present author.



Sodium hydroxide preparations do not "always" contain large amounts of chloride, as stated by Whitehorn. That made from the metal theoretically cannot and actually does not contain it, and that purified by alcohol contains only harmless traces. The technical sodium hydroxide which Folin recommends for nitrogen determinations of course always carries large amounts of chlorides and must not be used.

In respect of the manner of precipitating the proteins in samples of whole blood, it has been the author's experience that addition of the acid before dilution with water is an effective procedure and actual analyses have shown that the distribution of chloride is in no wise changed, if the blood be allowed to stand one hour as directed. It will repay one to do this if a nonprotein nitrogen determination be desired on the filtrate, because occasionally when filtered immediately, the liquid will foam during the digestion, making the results worthless and the specimen of blood cannot always be replaced.

It may also be mentioned that oxalate is preferred to citrate as an anti-coagulant because the latter interferes with the alkalimetry of the specimen should that be desired. The light green color obtained through its interaction with the ferric alum causes the pink color of the ferric sulphocyanate to appear brown but it does not interfere with the endpoint in the concentration advised.

The filtrates will, of course, always contain more or less tungstic acid. This serves a useful purpose by inhibiting the coagulation of the precipitated chloride until the mixture has been diluted to the mark and vigorously shaken. Obviously, any silver nitrate occluded under these conditions cannot influence the results of the titration, since the latter is carried out on aliquots. Silver chloride will coagulate upon shaking without addition of nitric acid and its failure to do so implies the presence of too much tungstic acid. The addition of nitric acid overcomes this but forms curds which can occlude more or less silver nitrate, and since Whitehorn does not employ aliquots of the filtrate, his method would theoretically be more likely to give high results than the method of the present author.

As evidence of the accuracy of his procedure, Whitehorn publishes a series of check determinations of chlorine in plasma, showing some of the results in agreement to within 0.01 mg., per c.c. It is difficult to understand how such close checks can be obtained unless a burette delivering one drop to 0.01 c.c., be used, since the formula given on p. 451 (l.c.) reads  $5.00 - \text{titer in c.c.} = \text{mgs. of chlorine per c.c. of blood or plasma}$ . Using the ordinary laboratory burette, delivering 0.05 c.c. to a drop any deviation from the theoretic figure must be at least  $\pm 0.05$  mg. per c.c.

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- <sup>2</sup>Whitehorn, J. C.: Simplified Method for the Determination of Chlorides in Blood or Plasma, *Jour. Biol. Chem.*, 1920-21, xiv, 449.

## THE METHYL ALCOHOL IN ARSPHENAMINE (SALVARSAN)\*

BY PHILIP ADOLPH KOBER, B.S., NEPERA PARK, N. Y.

SINCE Ehrlich's work on salvarsan, it was generally assumed that, since the arsenic content of the drug is 31.6 per cent and not 34.4 per cent as it ought to be for the pure dry drug, Ehrlich's preparation and those preparations made according to his formula or method, have two molecules of water of crystallization which would account for an arsenic content of 31.6 per cent as it is found on analysis. In 1918<sup>1</sup> while working at the New York State Health Laboratories, I tried to show that there really was no valid reason for assuming that there were two molecules of water in the drug, but that all the evidence pointed to the presence of a molecule of methyl alcohol, free or combined, in the finished marketed drug. In the same paper I described a method for preparing a methyl-alcohol-free arspenamine from aqueous solutions entirely.

Since then, from various sources, this work and the conclusion drawn from it has been disputed, and findings and conclusions of other investigators have been so different that we ought if possible account for these differences. That is the object of this paper. I also wish to discuss briefly the toxicologic effect of the methyl alcohol in the drug, and the properties of a methyl-alcohol-free arspenamine preparation.

The first criticism encountered was that a methyl-alcohol-free arspenamine was not a real arspenamine since it differed from the Ehrlich product in constitution and somewhat in physical properties. The assumption underlying this criticism was that Ehrlich made all of his fundamental work on spirochetes, *in vitro* and *vivo*, with arspenamine, which known as No. "606" is the *dihydrochloride of the salvarsan base*, the curative principle of all arspenamine and neoarsphenamine preparations. Therefore it was concluded that a methyl-alcohol-free arspenamine meant going into the field with a new drug of unknown therapeutic power!

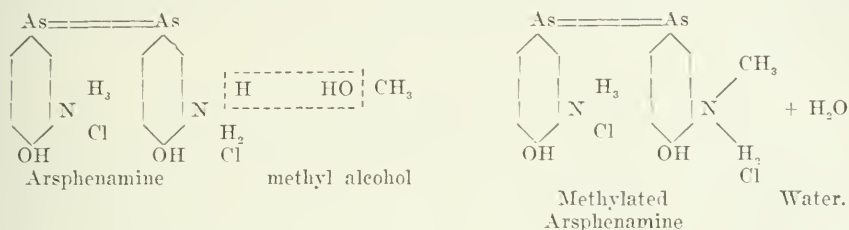
The fact is that Ehrlich<sup>2</sup> did not make his fundamental experiments with "606" but with No. 592, which is the salvarsan base, and therefore the arspenamine preparation which yields upon neutralization the pure and original base, is a true Ehrlich preparation, like upon which his fundamental work was based. The importance of a pure arspenamine base is also discussed in more detail below.

The second criticism was that after all there was no methyl alcohol present in Ehrlich's preparation or in preparations made according to his method. Meyer<sup>3</sup> from the laboratories of the H. A. Metz, Inc., reported that he had dried the Metz preparation on a large scale and found appreciable water,

\*From the Laboratories of the Kober Chemical Company, Inc., Nepera Park, N. Y.

and traces only of hydrochloric acid and methyl alcohol. Meyer was asked at the meeting when this statement of his was made, how he determined his methyl alcohol and the writer was informed that it was a trade secret. Even from the meagre data available it was evident that the solvents in the drug, after having dissolved the drug in water, were not distilled, but the drug dried more than usual and an attempt made to determine the volatile products.

The Metz product is claimed to be identical with the German and Ehrlich salvarsan and therefore made with methyl alcohol and ether. The only way for their product to give off water is by the methyl alcohol combining with arsphenamine forming methylated arsphenamine, and water produced as a result of the reaction with methyl alcohol, in accordance with the following:



Fargher<sup>4</sup> and Pyman of the Burroughs, Welleome & Co. Laboratories, London, also came to the conclusion that salvarsan contains no combined or free methyl alcohol. These authors made two combustion analyses on a sample of arsphenamine and found no increase in carbon content that would be in harmony with methyl alcohol present in the drug. However the hydrochloric acid content of these preparations was about 50 per cent below what arsphenamine is supposed to contain so that these two combustion analyses can have very little if any weight. These authors then dissolved some arsphenamine and distilled the resulting solution and got no more than traces of methyl alcohol. Whether here the methyl alcohol had been attached to the arsphenamine molecule as in reaction No. I, due to excessive drying and therefore methylation, cannot be proven here, but if the samples taken for combustion analysis are any criterion that is just what was done. These authors admit that when the drug is precipitated by means of acetone, even though it is no solvent for arsphenamine such as methyl alcohol is, the finished drug contains a whole molecule of acetone.

That arsphenamine made according to Ehrlich's directions does contain methyl alcohol is supported by the following facts:

(1) That Ehrlich<sup>5</sup> himself found methyl alcohol by qualitative as well as by quantitative methods in one of his batches.

(2) That Rieger<sup>6</sup> found a "strong Mulliken and Seudder" methyl alcohol "test on samples of German salvarsan, American salvarsan and Diarsonal."

(3) I have repeatedly found methyl alcohol and other organic solvents, in arsphenamine made by Ehrlich's method and even after a reprecipitation with hydrochloric acid, on distillation from an aqueous solution obtained a methyl alcohol test.

(4) Raiziss from the Dermatological Research Laboratories has admitted that arsphenamine contains methyl alcohol<sup>7</sup> and in a recent article<sup>8</sup> gives results which confirm my finding of alcohol in neoarsphenamine.<sup>9</sup>

(5) The drug can hardly have any water in it, when it is precipitated from absolute methyl alcohol and anhydrous ether, and must contain methyl alcohol or some other impurity to account for its low arsenic content.

Considering that all positive evidence is in support of methyl alcohol being present in arsphenamine made according to Ehrlich's method, and that negative evidence is accounted for by faulty or unsuited technic, there seems no doubt, then, that Ehrlich's salvarsan was contaminated with one molecule of methyl alcohol, either free or combined, depending upon whether the solvents and material were extremely anhydrous and whether the product was dried much or not.

### *The Toxicological Effect of the Methyl Alcohol*

The question arises what is the effect of the presence of a molecule of methyl alcohol. The amount of methyl alcohol is not large, being about 7 per cent or about 42 milligrams in a dose of 0.6 gram. What the direct toxicological effect of 42 milligrams of methyl alcohol is, when injected intravenously into the body, in the presence of arsphenamine, is not definitely known. Unless large amounts of the drug containing free methyl alcohol are frequently injected, no direct effect is likely to be felt, unless it is administered in cases of complications.

When the methyl alcohol is combined to the arsphenamine the effect is likely to increase the toxicity. Ehrlich and his collaborators<sup>10</sup> made methyl derivatives and found that they were very toxic and therapeutically not effective. Therefore the presence of combined methyl alcohol is apt to increase the toxicity and lower its therapeutic value.

Rieger<sup>11</sup> in his published work came to the following conclusion: "Commercial arsphenamine may contain an arseniurated methyl compound which decomposes either in the ampule or in solution, with liberation of arsenous oxide or a cacodyl-like substance. Some preparations betray the presence of the arsine by their garlie-like odor when dissolved; others develop it only after having stood in solution for hours. According to the amount that may have accumulated, the dosage and the idiosyncrasy of the patient, a reaction marked by fall in blood pressure, dyspnea and cyanosis may occur. Once the drug has been injected, the occurrence of subacute or chronic arsenic poisoning is determined by the margin that exists or may be made to exist between its elimination and its reduction by the tissues. There is evidence that this reduction to metallic arsenic occurs too readily for safety with present commercial preparations of arsphenamine."

Furthermore the technic involving methyl alcohol and ether is from a chemical point of view unsuited for treating a very sensitive and highly reduced substance like arsphenamine. Alcohol and especially ether with its peroxides are likely to act as organic catalysts, causing oxidation and other



changes in the constitution of the drug. This indirect effect of the presence of the methyl alcohol is in harmony with the toxicity of the drug, as found on the market. The German drug seems to have a tolerated dose of 60 mg. per kilo on white rats, the American product as it first appeared on the market had about the same toxicity, and owing to increased skill in handling the product, especially in keeping the air away and working at low temperatures, the toxicity of the American product has gradually decreased so that 100 mg. per kilo is the Standard of the U. S. P. H. Service today. But the fact is well known that manufacturers find from time to time that their product is too toxic to be used, without, however, knowing the direct cause of the trouble. This seems never to be the case if the arspenamine is prepared without the use of methyl alcohol and ether. I<sup>11</sup> found that I had no difficulty with the hydrochloric acid method of preparing arspenamines that were in every instance above the 100 mg. per kilo standard. Christiansen<sup>12</sup> working in Professor Reid Hunt's laboratory found that with the hydrochloric acid method he could get arspenamine which averaged 140 mg. kilo never being below 120 mg. kilo for albino rats. Some batches went as high as 160 mg. kilo. Trying the method for the first time M. Lewis I. Nurenborg, of the Massachusetts Health Department Laboratory found that he could get 130 to 140 mg. kilo toleration from reworking old and toxic batches.

#### *The Properties of a Methyl-Alcohol-Free Arspenamine*

It was found that when arspenamine was made especially pure and not excessively dried, it did not dissolve in cold water quite so readily, owing to its having an increased tendency to gelatinize when first moistened with water. Thus, Ehrlich's<sup>13</sup> first products dissolved slowly! The products first made by Raiziss<sup>14</sup> in the Dermatological Laboratories dissolved slowly, so that boiling water was called for in the printed directions accompanying the drug. The products made by me with the hydrochloric acid method<sup>15</sup> also dissolved slowly. Fagher and Pyman<sup>16</sup> also found that their purest arspenamine, made for scientific study was of slow solubility. Christiansen's purest products, some with a tolerated dose of 60 per cent above the Government standard dissolved slowly. In short, arspenamine made pure by three different methods reveals a drug with a tendency to produce a slowly dissolving gel in cold water and rapidly dissolving gel in hot or warm water, so that we are forced to the conclusion that the slow solubility in cold water is a characteristic of the pure arspenamine. Therefore we should not change the chemical composition of the drug for the purpose of hastening solution. The gain of a minute or two of time in dissolving is not commensurate with the loss of chemical constitution of the drug and its resultant danger to the host and loss of therapeutic power.

After the arspenamine has been dissolved in water, it must be properly and quickly neutralized, so that air is not allowed to act long on the alkalized solution, as Roth<sup>17</sup> has shown that exposure of the alkaline solution to the air results in an increase of toxicity of the drug.

## SUMMARY

The properties of a pure arsphenamine are:

- (1) It gelatinizes in cold water.
- (2) Its gel dissolves in warm water.
- (3) Its color is a light straw in solution.

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- <sup>5</sup>Berichte der deutschen Chem. Gesellschaft, 1912, xlv, 756.
- <sup>6</sup>Jour. Lab. and Clin. Med., 1919, iv, 181.
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- <sup>8</sup>Raiziss: Jour. Biol. Chem., 1921, xlii, 209.
- <sup>9</sup>Kober: Science, 1920, lii, 414.
- <sup>10</sup>Bertheim, A.: Berichte der deutschen Chem. Gesellschaft, 1912, xlv, 2135.
- <sup>11</sup>Loc. cit.
- <sup>12</sup>Christiansen: Jour. Am. Chem. Soc., 1920, xlii, 2402.
- <sup>13</sup>Ehrlich and Hata: Loc. cit.
- <sup>14</sup>Printed circulars of Dermatological Laboratories.
- <sup>15</sup>Kober: Loc. cit.
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- <sup>17</sup>Public Health Reports, U.S.P.H.S., xxxv, No. 38, pp. 2205-2210.

## BLOOD COUNTS WITH OXALATED BLOOD\*

BY NANCY VARBROUGH, CHARLOTTE, N. C.

IT IS often very difficult for the physician who visits out-of-town patients to find the ways and means of having his blood counts made. In order to help solve this problem we have worked out a plan whereby the blood can be procured by the visiting doctor and brought back to the home base for examination. The method of procedure is very simple and one that every practicing physician can carry out.

## MATERIALS

1. A sterile 5 c.c. syringe.
2. A sterile tube containing dried potassium oxalate. Potassium oxalate prevents clotting and four drops of a 20 per cent solution, dried in the hot air sterilizer, will preserve 30 c.c. of blood.

## METHOD

Draw blood from the vein and immediately place in the oxalate tube and shake thoroughly for one minute: cork securely and take to the laboratory for counting.

In this laboratory blood was taken as described above and at the same time counts were made on blood taken from the ear or finger of the same patient.

\*From the Laboratory of the New Charlotte Sanatorium.

Counts on the oxalated blood were approximately identical with those taken from the finger. Complete blood counts can thus be made satisfactorily on oxalated blood. Below a few exact figures are cited.

CASE 1. Leucocyte count on fresh blood, 9,400, on oxalated blood, 8,700.		
CASE 2.	<i>Fresh blood</i>	<i>Oxalated blood</i>
Leucocytes	18,000	17,700
Red cells	3,328,000	3,464,000
Hemoglobin	45 %	45 %
CASE 3.	<i>Fresh blood</i>	<i>Oxalated blood</i>
Leucocytes	7,500	7,500
Red cells	4,352,000	4,072,000
Hemoglobin	90 %	90 %
Differential count (200 cells counted)		
Polynuclears	67 %	62.5 %
Mononuclears	28	32.5
Eosinophils	5	4.5
Basophils	0	0.5

From the above figures it can easily be seen how nearly identical are the counts on fresh and oxalated blood. Two people making counts on fresh blood would probably vary as much in their respective reports. Oxalated blood will keep at least three days, giving correct counts at the end of this time. The following precautions are necessary:

1. Blood must be forced immediately out of the syringe into the oxalated tube.
2. The blood must be shaken thoroughly for at least one minute. Any clotting whatsoever destroys the accuracy of the count.
3. The blood must again be shaken thoroughly before counts are made.

## AN APPARATUS FOR THE ESTIMATION OF CATALASE\*

BY WM. H. WELKER, A.C., PH.D., CHICAGO

SINCE catalase had been assumed to run parallel with oxydase, and consequently supposed to be an index of the oxidative activity in the organism or in various portions of the organism, it seemed desirable to try the effect *in vivo* of a reagent such as potassium cyanide, which according to the generally accepted view, diminishes oxidation.

At the time this work was begun, most of the investigators studying catalase had been doing the shaking by hand and some of them were not even using leveling bulbs in connection with the measuring tubes, and consequently were introducing large experimental errors. It seemed desirable to construct a machine by which the mixture of blood and hydrogen peroxide could be shaken uniformly and by which a series of determinations could be carried out at one time, with provision for reasonably accurate measurement of the gas liberated.

\*From the Laboratory of Physiological Chemistry, College of Medicine, University of Illinois.

The machine shown in Fig. 1 consists essentially of a support of three-eighth-inch pipe from which a board is suspended by four brass rods. The eccentric on the shaft of the driving wheel is connected with this suspended board by a rod having a flexible joint at the board end. The bottles used in the machine are 500 c.c. salt-mouth bottles, ground for glass stoppers. These bottles are supported on the board by means of copper cans fastened to the board, and are held rigidly in the cans by means of thermos bottle springs. Ordinary rubber stoppers proved unsatisfactory for closing the bottles. This difficulty was overcome by having made to order, some double length stoppers. These can be seated so firmly in the bottle necks that no gas leakage occurs. For measuring the liberated gas each of the bottles is connected with

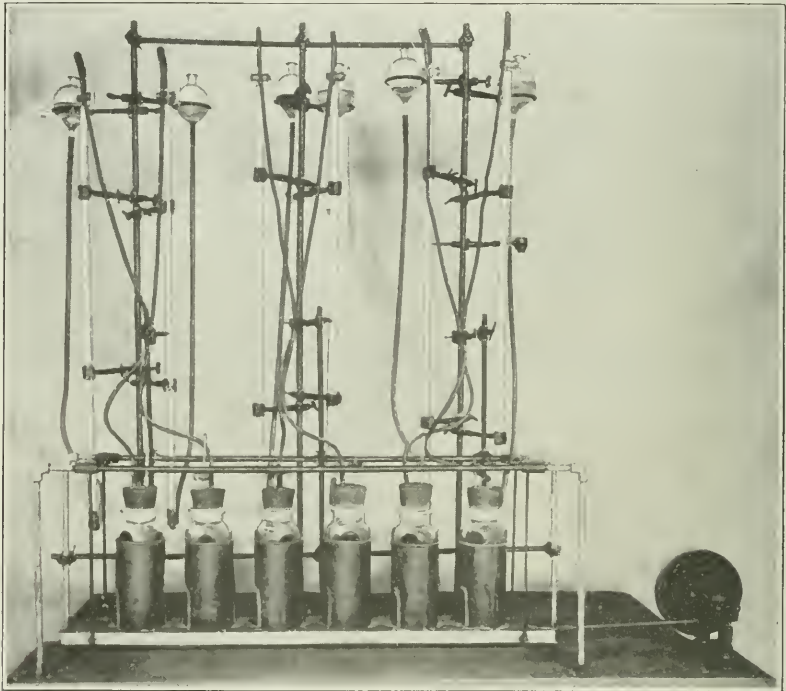


Fig. 1.

the top of a gas measuring tube by a rigid wall, rubber tube. To the bottom of the gas measuring tube is connected a leveling bulb. The six gas measuring tubes with their leveling bulbs are supported by a special stand built for the purpose. Aluminium caps from screw-cap vials are used as containers for the blood.

The mode of operation is as follows: 75 c.c. of diluted hydrogen peroxide (equal volumes of hydrogen peroxide and water) are measured into each of the bottles. The samples (usually .5 c.c.) of blood are then accurately measured into the aluminium caps. These are floated on the surface of the hydrogen peroxide by means of a pair of tongs, made by straightening out the curved ends of a pair of crucible tongs. The stoppers are then placed firmly



into the bottle necks. The stopcocks on the bottles are opened and the level of the liquid in the gas measuring tube adjusted so that the reading is zero. The stopcocks on the bottles are then closed and the shaking is commenced. In the work carried on with this machine, readings have been taken every five minutes, at atmospheric pressure.

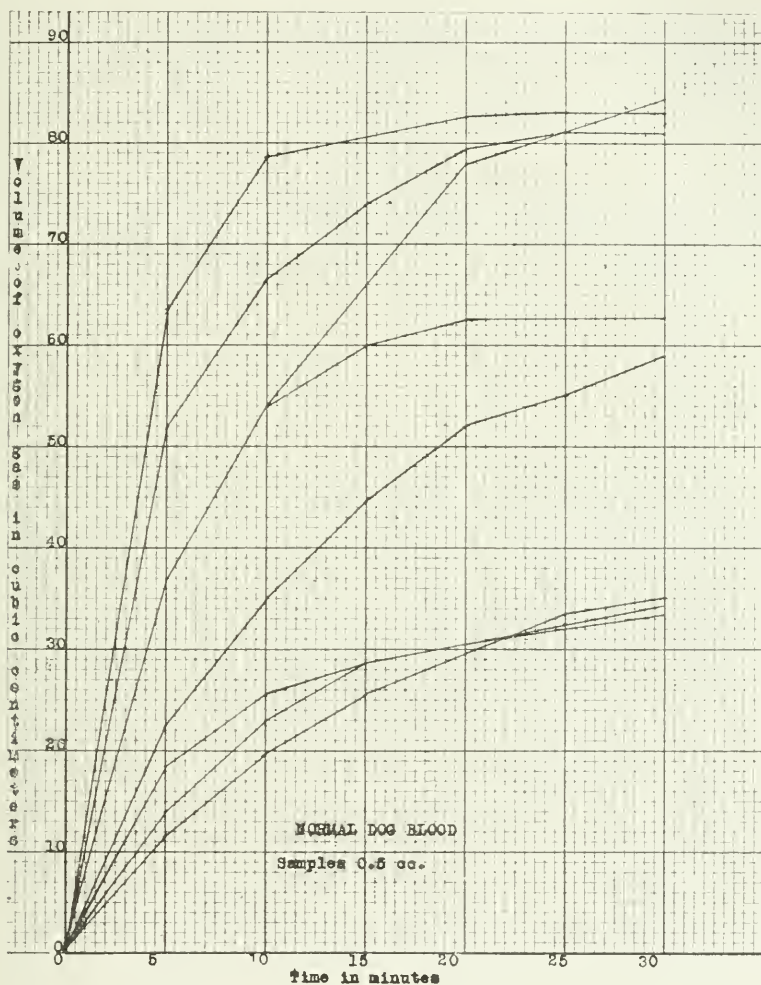


Fig. 2.

Fig. 2 shows a series of curves obtained from normal blood of dogs. These curves are based on results corrected for temperature, barometric pressure, and aqueous tension.

I am indebted to Dr. T. B. Magath for assistance in working out some of the details of the apparatus.

# THE ESTIMATION OF INORGANIC PHOSPHORUS IN BLOOD PLASMA BY THE METHOD OF BELL AND DOISY\*

BY BURTON A. MYERS AND MARIAN C. SHEVKY, SAN FRANCISCO, CAL.

WE HAVE met with certain difficulties in the practical application of Bell and Doisy's<sup>1</sup> recently published method to the determination of inorganic phosphorus in blood plasma which we believe are worthy of note, because their removal may broaden the usefulness of a method which has the

TABLE I  
KNOWN CONCENTRATIONS OF PHOSPHORUS READ AGAINST A STANDARD CONTAINING 0.5 MG. P PER 100 C.C.

ACTUAL P CONCENTRATION MG. PER 100 C.C.	P CONCENTRATION FOUND MG. PER 100 C.C.	ERROR %
2.00	1.73	-13.5
1.75	1.56	-10.9
1.50	1.35	-10.0
1.25	1.08	-14.0
1.00	0.85	-15.2
0.75	0.67	-10.7
0.25	0.25	± 0.0

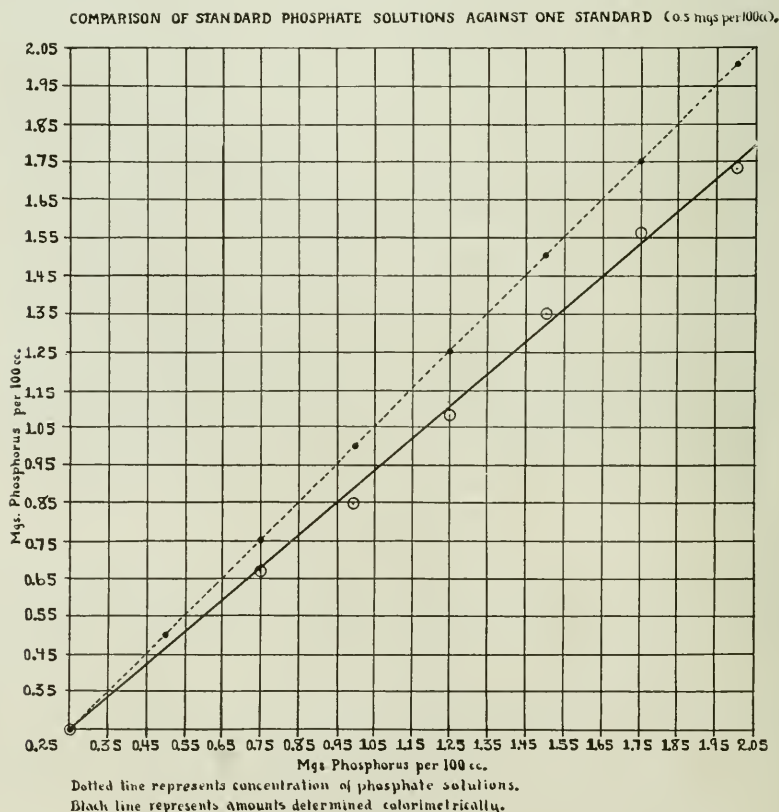


Fig. 1.

\*From the Laboratory of the Division of Medicine, Stanford University Medical School, San Francisco, Calif.

great advantage of comparative simplicity. We were led to the study on account of a failure to recover phosphorus added to plasma.

A series of solutions of varying concentrations of acid potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) were prepared which corresponded to the range of concentration in which inorganic phosphorus is known to exist in protein-free plasma filtrates. The solution containing 0.5 mg. of phosphorus per 100 c.c., which is probably near the average phosphorus concentration of plasma filtrates, was taken as the standard. Results were obtained as shown in Table I and Figure 1.

The only solution correctly determined was the one which was weaker than the standard, and all the others showed considerable minus errors.

TABLE II  
KNOWN CONCENTRATIONS OF PHOSPHORUS READ AGAINST A STANDARD CONTAINING 0.5 MG. P PER 100 C.C. AFTER DILUTING EACH WITH WATER IN THE AMOUNT THEORETICALLY REQUIRED TO MAKE THE COLORS EQUAL

ACTUAL P CONCENTRATION MG. PER 100 C.C.	P CONCENTRATION FOUND MG. PER 100 C.C.	ERROR %
2.00	1.38	-36.0
1.75	1.12	-31.5
1.50	1.02	-32.0
1.25	0.87	-30.5
1.00	0.73	-26.7
0.75	0.61	-19.5

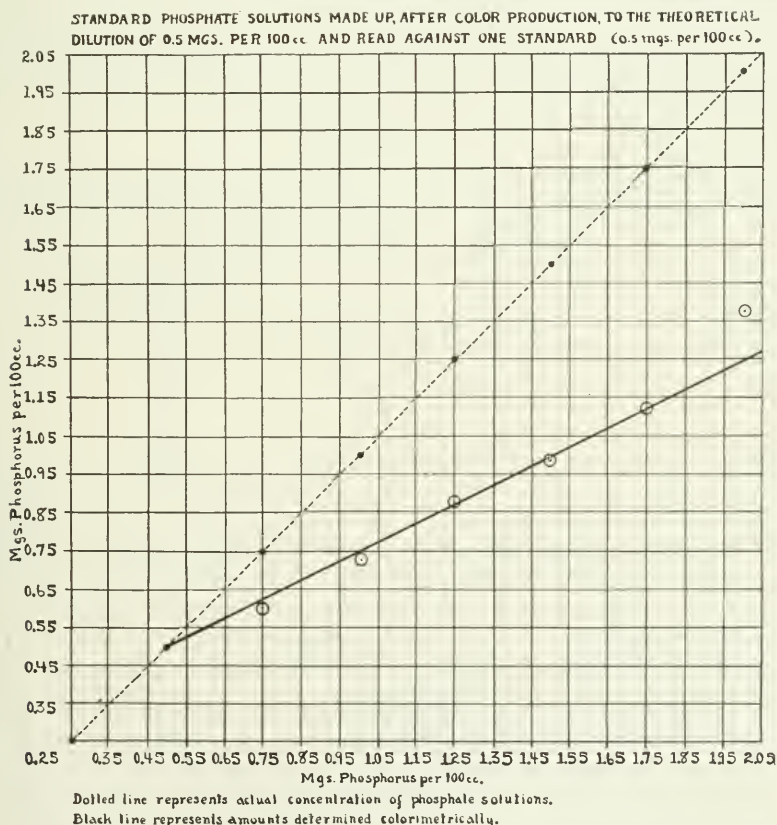


Fig. 2.

In order to obviate the difficulty of comparing colors varying considerably in density, the same procedure was repeated except that after the development of the blue color all the solutions were diluted with water in amounts which should theoretically have made all the colors equal. The results are given in Table II and Fig. 2.

It is clear from these figures that the amount of the blue color which appears is not directly proportional to the amount of phosphorus actually present, and that it was this fact which accounted for our inability to recover all the phosphorus we added in our experiments with plasma.

Bell and Doisy state: "It may be necessary to use a stronger standard or less filtrate in bloods containing much phosphorus. The proper standard and amount of filtrate may be roughly estimated before the determination is made." But there are no data given as to the degree of permissible difference between the standard and the unknown, and it is clear that this is an essential point to establish before the method can have any practical application. It has been noted by Greenwald<sup>2</sup> that the phosphorus in the blood serum of normal persons may range from 1.0 to 7.0 mg. of phosphorus per 100 c.c. It is also possible in normal individuals to increase the concentration of phosphorus beyond these limits.<sup>3</sup> Marriot and Howland<sup>4</sup> found a great increase in the inorganic phosphates in nephritis with acidosis, extending from 8.0 to 23.0 mg. of phosphorus per 100 c.c. of blood serum.

We have found that known phosphate solutions could be estimated with a fair degree of accuracy when the concentration of the standard was not more than 0.25 mg. of phosphorus per 100 c.c. greater than the unknown.

TABLE III

KNOWN CONCENTRATIONS OF PHOSPHORUS READ AGAINST DIFFERENT STANDARDS  
EACH CONTAINING 0.25 MG. PHOSPHORUS MORE THAN THE CON-  
CENTRATION OF PHOSPHORUS EXAMINED

STANDARD MG. PER 100 C.C.	ACTUAL PHOSPHORUS CONCENTRATIONS MG. PER 100 C.C.	PHOSPHORUS CONCENTRATION FOUND MG. PER 100 C.C.	ERROR %
2.00	1.75	1.79	+2.0
1.75	1.50	1.49	-0.5
1.50	1.25	1.24	-0.8
1.25	1.00	0.99	-0.6
1.00	0.75	0.72	-3.7
0.75	0.50	0.53	+5.0

Table III and Fig. 3 show that in working with unknown solutions, it will be necessary to have a series of standard solutions, so that a standard may be selected which contains not more than 0.25 mg. of phosphorus per 100 c.c. more than the solution of which the phosphorus content is to be determined.

Another difficulty arose in work with rabbit plasma. In some instances, but not in all, little or no color was produced when the usual amounts of molybdic acid and hydroquinone solutions were used. It was found, however, that in all cases the color appeared, when to 5 c.c. of the plasma filtrate (1:5 dilution), 1.5 c.c. of molybdic acid and 3 c.c. of hydroquinone solution were added. Since this variation in the amount of reagents used had no effect on the color pro-



duction, either in phosphate solutions or in plasma, we believe that it is safer to adopt this modification. This nonproduction of color is not peculiar to rabbit plasma, for we have seen it occur with one human plasma, though never in beef serum.

A small series of determinations of inorganic phosphorus in filtrates from plasma and beef serum before and after the addition of known amounts of

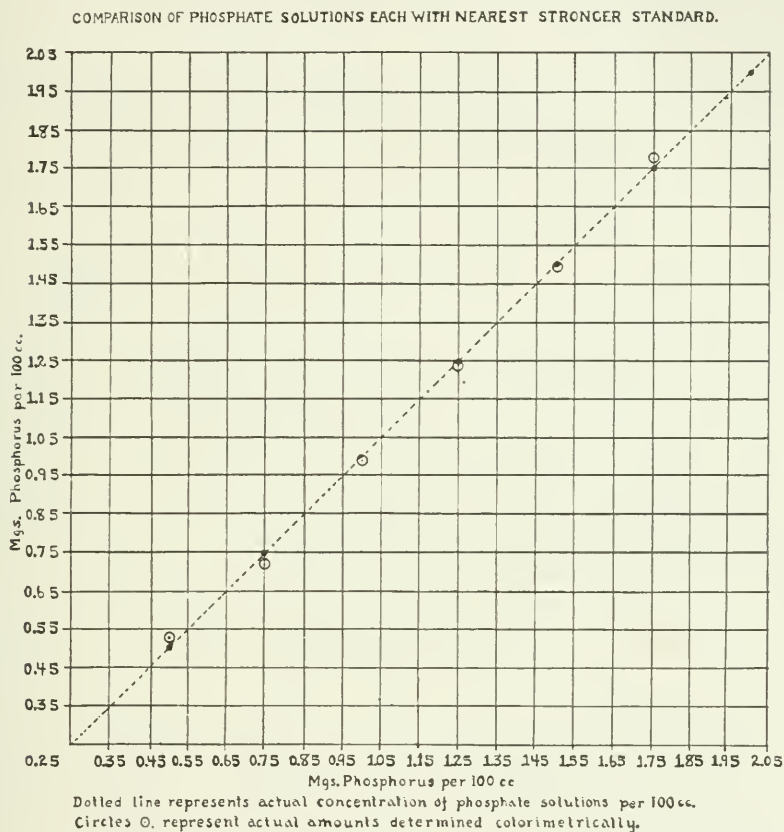


Fig. 3.

phosphorus showed that under these conditions the error of the method ranged from 2.3 per cent to 10.0 per cent with an average of 5.8 per cent. In every case the standard used contained phosphorus in an amount which did not exceed that in the unknown by more than 0.25 mg. of phosphorus per 100 c.c.

#### CONCLUSIONS

1. A series of standards must be prepared so that in each determination one may be selected which contains phosphorus in an amount which does not exceed that in the unknown by more than 0.25 mg. per 100 c.c.
2. In many rabbit plasmas and in some human plasmas larger amounts of molybdic acid and hydroquinone solutions must be added.
3. A small series of determinations of phosphorus in plasmas to which

known amounts of phosphorus were added indicated that the average error was about  $\pm 6.0$  per cent with a maximum of 10.0 per cent.

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### NOTE ON THE DIFFERENTIAL STAINING OF THE GRANULES IN DIPHTHERIA AND OTHER BACILLI\*

BY ALBERT G. NICHOLLS, M.D., D.Sc., HALIFAX, N. S.

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THE method of demonstrating the granules in bacteria described here was accidentally discovered by the writer in a moment of inadvertence. While staining a number of slides of bacteria by the Neisser double staining method and by Gram, the preparations became mixed. The error was discovered after certain of the slides had been treated with Neisser No. 1 and then with Gram's iodine solution, when examined at this stage it was found that granules in diphtheria bacilli were exceedingly well brought out, and it only required a little experimenting to produce a method of granule staining which had the merits of simplicity, certainty, and effectiveness. The procedure is as follows:

Stain films, fixed by heat, with Neisser's Staining Fluid No. 1. This consists of Methylene Blue, 1 gram; 96 per cent alcohol, 20 c.c.; glacial acetic acid, 50 c.c.; and distilled water, 950 c.c. This mixture is allowed to remain on the films for thirty seconds. Wash in water. Apply Gram's iodine solution for ninety seconds. Wash in water. Apply watery solution of Saffranin T one-half per cent for thirty seconds. The granules appear black on a pink background. The picture is sharp, the contrast excellent. The method is applicable to any bacteria which contain granules, and shows up the granules in diphtheria bacilli exceptionally well.

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# The Journal of Laboratory and Clinical Medicine

VOL. VII.

DECEMBER, 1921

No. 3

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Washington, D. C.

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## EDITORIALS

### *Arsphenamine and Neoarsphenamine Dosage*

WHEN salvarsan was introduced into the medical world, the details of its administration had been already worked out. Ehrlich, after having chemically perfected the spirocheticide, had had its action tested on thousands of individuals in several of the best German clinics, so that when the treatment came into general use the experimental phase had been passed, and the dosage and manner of administration had already been worked out in detail. In this way unfortunate accidents in the hands of inexperienced physicians were to a great extent avoided.

Nevertheless there have been several important and valuable changes in the method of arsphenamine therapy during the last ten years. In the treatment of syphilis, it was soon discovered that the "therapia sterilisans magna," which had been satisfactorily employed in animals could not be reproduced in man. Our inability to destroy all of the spirochetes in the body with one massive intravenous medication has rendered it necessary to administer repeated doses. The question of dosage in the treatment of syphilis has therefore become extremely important.

Ehrlich in his original work on trypanosomes, recognized that in those cases not entirely cured by the first treatment, relapses were even more difficult to cure. Treatment which had been unsuccessful in a first attempt was usually unsuccessful on repetition. Repeated treatments, none of which cured entirely, were often found to have progressively less influence on the activity of the infecting parasite. A condition of "Festigkeit," increased tolerance or resistance to the action of the drug, had been produced.

The possible development of "drug-fastness" by the infecting agent in individuals undergoing antisyphilitic treatment, is now recognized as an important feature and a phenomenon to be avoided if possible. In the case of trypanosome infection Ehrlich found not only that the parasite became resistant to the chemical action of the drug, but also that the increased tolerance persisted after transplantation into another host. A strain resistant to atoxyl in the mouse maintained its high tolerance to the drug throughout 46 subsequent passages through rats. Eventually, however, the parasite tends to regain its original sensitiveness to the drug in question. The possible development of a race of spirochetes resistant to the action of arsphenamine, one for which we must find some new or stronger method of therapy, must be considered.

A contribution of considerable significance has been made recently by Bronfenbrenner and Schlesinger,<sup>1</sup> who found that minute doses of arsphenamine, instead of inhibiting the progress of the spirochetal infection, actually caused the disease to progress more rapidly and more extensively in the animals so treated than in other animals similarly infected but not treated at all. Bronfenbrenner and Noguchi<sup>2</sup> found some time ago that minute amounts of arsphenamine present in culture media stimulated the growth of spirochetes. Very minute quantities of arsphenamine appear therefore to be of less value than no treatment at all. The phenomenon appears analogous to that found in the treatment of cancer by the roentgen ray, where small doses stimulate the cells to proliferation while larger doses destroy. Bronfenbrenner and Schlesinger point out that this stimulative action of minute doses of arsenical compounds is not to be confused with the development of increased tolerance, but is characterized by an actual increase in the rapidity of multiplication of the parasites.

The dosage used by these investigators is of course extremely small and it does not follow from their report that the occasional small doses used therapeutically in the treatment of syphilis have a stimulating action. It is quite probable that even these small doses are sufficiently large that they inhibit multiplication. This is indicated by the customary diminution in the intensity of the positive Wassermann reaction.

Brown and Pearce<sup>3</sup> have shown in a recent article that insufficient treatment with arsphenamine or neoarsphenamine may alter the immunologic status in experimental animals to such an extent as to favor the development of a second or superimposed infection without a cure having been accomplished for the first. They inoculated rabbits, and after the development of the primary lesion, treated the animals with single massive doses of arsphenamine or neoarsphenamine. The primary lesions as a rule retrogressed and in some instances disappeared. Reinoculation, at a time when the drug was no longer present in the body in sufficient



amounts to produce an effect, and when the original primary lesion had practically disappeared, produced in most of the rabbits a second primary lesion at the site of reinoculation. In the majority of the animals the original infection had not been entirely destroyed and as the original parasites once again commenced to multiply, chancres developed both at the site of the original inoculation and the site of reinoculation.

It has long been held that individuals once infected with the spirochete of syphilis are immune to reinfection as long as the germ remains active in the body. Reinfection usually is assumed to be evidence that the patient had been cured of the former infection. The work of Brown and Pearce shows quite conclusively that experimentally at least, an individual may become reinfected with syphilis even though the original invaders are still living in his tissues. Similar phenomena have occasionally been reported in man.<sup>4</sup>

How frequently the factors discussed above will become of importance in the treatment of syphilis is uncertain. Certainly the standard doses in use in the large clinics have proved their worth. But that they are the ideal dosage does not follow. There is a tendency with many, to give relatively smaller doses of arsphenamine or neoarsphenamine than formerly. Past experience and recent laboratory observations appear to be of accord in indicating that the dosage in antisypilitic treatment should be as high as safety permits.

Mehrtens<sup>5</sup> has recently reported on the administration of neoarsphenamine by the rectum. He does not prefer this method to intravenous therapy but suggests its use in cases where the latter is difficult or impossible, as in children or in individuals in whom it is impossible to enter the vein. Assuming the value of high arsenical concentration in the destruction of spirochetes one may conclude from Mehrten's tablets that rectal administration is not as desirable as intravenous treatment. Five minutes after the intravenous administration of 0.6 gm. of arsphenamine, arsenic is present in the blood in a concentration of 0.97 mg. per 100 c.c. By the end of an hour it has fallen to 0.077 mg. per 100 c.c. One hour after the administration of 4 gm. of neoarsphenamine by rectum, there is 0.0015 mg. of arsenic in each 100 c.c. of blood. Absorption from the rectum is slow and it requires 12 hours before the blood has attained a maximum concentration of 0.025 mg. per 100 c.c. From the 6th to the 14th hour after administration the concentration in the blood equals or exceeds that after intravenous administration but this amount is exceedingly small as compared with that to be found immediately after injection directly into the vein. It should also be borne in mind that the dose of neoarsphenamine by rectum is 4 gm. as contrasted with 0.6 gm. of arsphenamine by the vein. Mehrtens remarks that the actual quantities of arsenic present in the blood after administration by rectum is at all times very small.

He also observed the amount of arsenic excreted in the urine both after intravenous administration of 0.6 gm. of arsphenamine and following 4 gm. of neoarsphenamine by rectum. The amount of arsenic excreted in the urine during the first twelve hours was over three times greater following rectal administration than after intravenous administration. Very much the same ratio was found during the second 12 hour period. Even two days after

treatment, nearly three times as much arsenic was still appearing in the urine. Three days and a half after administration, arsenic was still present following both methods, but in rapidly diminishing amounts. Following a very much smaller dose intravenously, arsenic appears to have persisted in the urine nearly as long as after a massive dose through the rectum.

Mehrtens states that he observed no signs of toxicity after the large dose other than occasional attacks of vomiting, and in two cases, puffiness under the eyes. This latter observation might be associated with the added burden to the kidneys. Where there is renal impairment this method should probably be avoided.

Rectal treatment would theoretically appear inferior to intravenous therapy, first because the concentration of arsenic in the blood and probably also in the body tissues never becomes as high, and, second, because the kidneys are placed under an unusual strain. It should be repeated here that Mehrtens does not recommend this method as an improvement or even as a substitute for intravenous medication.

Adverse theoretical considerations are without weight in the presence of demonstrated clinical results. If new methods show better clinical and serologic results, they must be accepted as improvements. If these improvements are not logical in view of existing postulates, the postulates must be changed. So far, however, modifications of the usual intravenous technic have not proved their greater worth. Mehrtens recognizes the difficulty in estimating clinical results. He also points out that the subjects treated were chiefly old neurosyphilitics in whom improvement under any form of treatment is neither rapid nor satisfactory. He does believe, however, that serologic improvement was at least as rapid by the rectal method as following intravenous administration.

Minet<sup>6</sup> claims truly astonishing therapeutic results from the daily subcutaneous injection of 0.15 gm. of neoarsenobenzol. If the important factor in the care of syphilis is prolonged saturation rather than massive dosage, both the technic of Mehrtens and that of Minet become entirely logical. This possibility will be discussed shortly. Probably both factors play a part.

The standard intravenous dose of arsphenamine is 0.6 gm., that of neoarsphenamine 0.9 gm. These quantities appear to be sufficiently large for satisfactory therapeutic results and yet are not so large that they cause untoward results. They contain about equal amounts of arsenic and are supposed to have equal therapeutic value. The majority of critical observers have found, however, that there is a difference in the effectiveness of the two drugs when given in these doses. Most syphilologists have concluded that arsphenamine produces prompter results and is less liable to be followed by relapse. The popularity of the second preparation is due to the greater ease of administration, a factor which should play a less important rôle than it actually does. In nearly all of the armies, during the War, syphilis was treated with neoarsphenamine. This was undoubtedly due to the greater ease, and shorter time required for administration, thereby facilitating the treatment of larger numbers of individuals, and to the fact that considerably less apparatus was required. Rightly or wrongly this has given an official sanction to the use of neoarsphenamine.

Considerable experimental work has been done within recent years to determine if possible the comparative value of the two drugs. Schamberg, Kolmer and Raiziss,<sup>7</sup> working with trypanosomes, found that the trypanocidal activity of arsphenamine was 1.74 times that of neoarsphenamine. Castelli and others, working on trypanosomiasis, spirillosis, and rabbit syphilis reached similar conclusions, finding that the activity of the former was between 1.5 and 1.78 times that of the latter. If we are to accept these results we should conclude that therapeutically 0.6 gm. of arsphenamine is not equivalent to 0.9 gm. of neoarsphenamine, but rather to 1.05 gm. The therapeutic activity of 1.05 gm. of neoarsphenamine is equivalent to that of 0.6 gm. of arsphenamine.

The trypanocidal dose of arsphenamine (*dosis therapeutica*) is 4.564 times less than the highest tolerated dose, (*dosis tolerata*). On the other hand the therapeutic dose of neoarsphenamine is 6.35 times smaller than the highest tolerated dose. The latter is therefore, a safer drug, the margin of safety between the two doses being distinctly greater. This is true even if 1.05 gm. instead of 0.9 gm. of neoarsphenamine should be used.

Voegtlin and Smith<sup>8</sup> have studied the same problem from a different angle and have reached similar conclusions. They ascertained the minimum effective dose and the minimum lethal dose in trypanosome infected animals. The minimum effective dose is that dose which will destroy practically all trypanosomes in the circulating blood. It is not a curative dose. A fairly sharp threshold exists for this dose, below which the drug has comparatively little trypanocidal activity. A minimum concentration of the drug appears necessary for the destruction of the majority of parasites present in the blood stream. The dose, below which the drug fails to destroy the parasites, has been found to be fixed partly by the reaction between the drugs and the parasites, and partly by the rate at which the drug is absorbed by the tissues of the host. Subeffective doses cease to act, not when they have killed a certain number of parasites, but when absorption by the host has lowered the concentration below the threshold. Voegtlin and Smith believe that a relapse can be avoided only by giving considerably more than the minimum effective dose. The question both with arsphenamine and with neoarsphenamine is, how high over the M. E. D. may we go without danger of reaching the minimum lethal dose?

The average M. E. D. for arsphenamine is 3.1 gm. per kilo body weight; for neoarsphenamine, 3.4. These two doses are approximately the same. The M. L. D. on the contrary is 53.2 for arsphenamine and 96.5 for neoarsphenamine. Once again we see a wider margin of safety in the latter drug. Voegtlin and Smith designate as the "therapeutic ratio," the ratio  $\frac{\text{M. L. D.}}{\text{M. E. D.}}$ . For arsphenamine this is 17.2, and for neoarsphenamine 28.4. The higher the ratio the less danger is there in administering the drug in amounts greater than the M. E. D.

These experimental observations coincide with clinical knowledge that toxic reactions occur less frequently after large doses of neoarsphenamine than after corresponding doses of arsphenamine.

Schamberg, Kolmer and Raiziss<sup>9</sup> have made a study of the relative toxicity of the two drugs. They found that for rats and mice neoarsphenamine is about



2.4 times less toxic than is arsphenamine. They conclude that, as far as is indicated by intravenous experiments on rats, the usual dose of 0.6 gm. of arsphenamine is about 12 times less than the highest tolerated dose, while 0.9 gm. of neoarsphenamine is 19 times less than the highest tolerated dose for the latter drug. Here again the conclusion is that larger amounts of neoarsphenamine are of less danger than are proportionate amounts of arsphenamine.

It is well known that neoarsphenamine may be given with safety at shorter intervals than arsphenamine. Will it not be true then, that if the former drug is administered in slightly larger doses and somewhat more frequently, say 0.9 gm. thrice weekly, equally good therapeutic results will be obtained? This raises a question as to whether the difference in therapeutic results is due merely to a difference in dosage or whether it depends on a chemical difference between the two drugs.

Swift<sup>10</sup> studied the rate of absorption of arsenic from the tissues after the intramuscular injection of both arsphenamine and neoarsphenamine. His work was done on rabbits. At the end of one week more than twice as much of the neoarsphenamine had been absorbed as of the arsphenamine. Lockemann<sup>11</sup> found that neoarsphenamine was excreted more rapidly. The latter drug is apparently absorbed more rapidly after local injection and is eliminated more rapidly from the body after absorption. Voegtlin and Smith suggest that arsphenamine is probably retained longer in the body than is neoarsphenamine because it is precipitated at the hydroxyl-ion concentration of the blood and therefore must be temporarily fixed in the tissues in a nonreactive state. This insoluble form is probably slowly oxidized in the body to the parasitocidal oxide and is gradually distributed as such through the blood stream. Neoarsphenamine on the contrary is not precipitated at the hydroxyl-ion concentration of the blood and its oxidation and elimination therefore proceed more rapidly. It is probable that this hypothesis represents the actual state, but it does not necessarily follow that this is the entire or the correct explanation for the therapeutic differences.

It is a curious fact that, experimentally, neoarsphenamine is usually more efficacious than is arsphenamine. But the laboratory observations are usually carried out on trypanosome infections or spirillosis, in which the parasite is predominantly in the blood stream. Here the relative rates of excretion are of less importance than in human syphilis. In the latter case it is important to reach the deep-seated foci, and this requires time. Too rapid excretion would prevent the drug from reaching deep-seated foci in the tissues.

Brown and Pearce have, however, observed that in rabbit syphilis neoarsphenamine is almost always more efficacious than is arsphenamine.

Schamberg<sup>12</sup> concluded from comparative studies that the addition of the formaldehyde sulphonylate group found in the neoarsphenamine, may lessen the affinity of this drug for the protoplasm of the parasite, but at the same time lessening the affinity for the body proteins to an even greater extent. This hypothesis would explain both the diminished therapeutic effect and also the decidedly lessened toxicity. Another phenomenon bearing on relative toxicity is the observation by Schamberg that arsphenamine in practically all concentrations hemolyzes red blood cells *in vitro*, while neoarsphenamine does not do so in



any concentration clinically employed. Schamberg has concluded that arsphenamine is more active therapeutically but that this advantage is balanced by the much higher tolerated dosage of neoarsphenamine and by the fact that the latter is less likely to cause biochemical disturbances in the blood and the tissues.

A point of great practical interest is that recently made by Roth<sup>13</sup> who has found that the shaking of alkalized aqueous solutions of arsphenamine and neoarsphenamine in the air for 60 or even 30 seconds, increases greatly the toxicity, probably by oxidation. This knowledge should lead to caution in the preparation of solutions for use in treatment.

The preceding review indicates fairly clearly the unsettled status of arsphenamine and neoarsphenamine dosage. At present there is sufficient evidence to justify those who prefer to use the simpler technic of neoarsphenamine therapy, but only on the condition that sufficiently large and frequent doses be administered. With either method, mercury and the iodides should not be neglected. Therapeutic results will be the ultimate criterion as to the relative advantages of arsphenamine and neoarsphenamine. We will await with interest further reports from the clinic and from the laboratory not only because of the practical interest but also because of the present inconclusive literature on the subject.

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—W. T. V.

### *Hypersensitiveness to Foods as Cause for Abdominal Pain*

SEVERE abdominal pain, says Duke, is a symptom which is never taken lightly by a careful physician. It often indicates a severe illness; in fact it often indicates an emergency. But, says Duke, there are cases in which abdominal pain may simulate that arising in serious abdominal disease, but which is the result of hypersensitiveness to food. It has been known for a number of years that hypersensitiveness to foods may give rise to bronchial asthma and a condition which simulates hay fever; to urticaria, angioneurotic edema, purpura, eczema, and other dermatoses; to dyspepsia, gastrointestinal upsets associated with vomiting, diarrhea, griping pains in the abdomen, and mucous colitis; to an interesting syndrome of symptoms known as Henoch's purpura. With the latter conditions a patient may have severe abdominal pain.

But abnormal pain sometimes occurs alone and is the sole striking symptom of hypersensitiveness to a food. In such cases the symptoms may be misleading in diagnosis. It is such cases that Duke discusses. He has observed a number of patients who have been sensitive to one or more of the following foods: egg white, egg yolk, shad roe, lactalbumin, casein, beef, pork, honey, strawberries, lettuce, almonds, beans, onions, cabbage, rice, potatoes, tomatoes, paprika and pimento, and who have invariably had an attack of abdominal pain whenever they have eaten the foods to which they were sensitive. In the majority of cases pain appeared soon after ingestion of the food and lasted for three to six hours. In several cases it did not appear until several hours later and then it lasted much longer. In the majority of cases pain was associated with nausea and vomiting, less frequently with indigestion, bloating, diarrhea and mucous stools, and much less frequently with hives, angioneurotic edema and purpura.

The pain complained of was evidently the result of a reaction caused by contact between the gastrointestinal mucosa and the food to which it was sensitive, and this contact gave rise to gastrointestinal symptoms in much the same way that contact of the mucous membrane of the respiratory tract with a pollen to which it is sensitive gives rise to symptoms of hay fever or asthma. In other words the pain and other gastrointestinal symptoms seem fundamentally analogous in pathogenesis to the symptoms of hay fever and asthma. An attack of asthma can be brought on in a sensitive individual by a subcutaneous injection of the pollen to which he is sensitive. Analogous to this several patients had gastrointestinal pain after subcutaneous injections of an extract of the food to which they were sensitive.

Perhaps the most interesting part of Duke's communication is that in which he calls attention to the fact that nearly 50 per cent of his patients who showed food hypersensitiveness had demonstrable pathologic lesions in the alimentary tract or its appendages. These lesions were recurrent appendicitis, gall stones, duodenal ulcer, dense adhesions and extreme ptosis. So, it is suggested, that while allergy is primarily dependent upon an inherited constitution which renders a person susceptible of becoming hypersensitive to certain alien substances, it is also possible that an abnormality in the alimentary tract may be a contributory factor in the etiology. It may even be the starting point of an inheritance if one can accept inheritance of acquired characteristics.

The diagnosis in these cases was founded upon careful observation of the diet and the physical reactions of the patients; they were confirmed by cutaneous tests.

Duke's paper appears in the *Archives of Internal Medicine*, 1921, xxviii, 151. The most recent discussion of the subject of inheritance of acquired characteristics is contained in Bernard Shaw's "Back to Methuselah," Brentano, 1921.

—P. G. W.

### *The National Board of Medical Examiners*

THE National Board of Medical Examiners has just completed the first five years' work and with it the trial period of its usefulness. The principle which this Board has stood for, namely, the establishment of a thorough test of fitness to practice medicine which might safely be accepted throughout this country and abroad, has been widely accepted. Since this Board was organized by Dr. W. L. Rodman, in 1915, eleven examinations have been held. These examinations have been conducted on the plan of holding at one sitting, a written, practical and clinical test for candidates with certain qualifications, namely a four-year high-school course, two years of college work, including one year of Physics, Chemistry, and Biology, graduation from a Class A Medical School and one year's internship in an acceptable hospital. These examinations have covered all the subjects of the medical school curriculum and have been conducted by members of the Board with members of the profession resident in the place of examination appointed to help them. Such examinations have been held in Washington, Philadelphia, New York City, Boston, Chicago, St. Louis, Rochester (Minnesota) and Minneapolis. During the war a combined examination was held at Fort Oglethorpe and Fort Riley. There have been 325 candidates examined, of whom 269 have passed and been granted certificates.

Starting with the endorsement of the Council on Medical Education of the American Medical Association, American Medical College Association and various sectional Medical Societies, the recognition of the Army, Navy and Public Health Service Medical Corps of the United States and certain State Boards of Medical Examiners, the certificate is now recognized. Also by twenty states as follows: Alabama, Arizona, Colorado, Delaware, Florida, Georgia, Idaho, Iowa, Kentucky, Maryland, Minnesota, Nebraska, New Hampshire, New Jersey, North Carolina, North Dakota, Pennsylvania, Rhode Island, Vermont and Virginia, the Conjoint Board of England, the Triple Qualification Board of Scotland, the American College of Surgeons and the Mayo Foundation of the University of Minnesota.

There has been such a widespread demand for an opportunity to secure this Certificate by examination, that the Board has now adopted and will put into effect at once, the following plan: Part I, to consist of a written examination in the six fundamental medical sciences: Anatomy, including histology and embryology; Physiology; Physiologic Chemistry; General Pathology; Bacteriology; Materia Medica and Pharmacology. Part II, to consist of a written examination in the four following subjects: Medicine, including pediatrics, neuropsychiatry, and therapeutics; Surgery, including applied anatomy, surgical pathology and surgical specialties; Obstetrics and Gynecology; Public Health, including hygiene and medical jurisprudence. Part III, to consist of a practical examination in each of the following four subjects; Clinical Medicine, including medical pathology, applied physiology, clinical chemistry, clinical microscopy and dermatology; Clinical Surgery, including applied anatomy, surgical pathology, operative surgery, and the surgical specialties of the diseases of the eye, ear, nose and throat; Obstetrics and Gynecology; Public Health including sanitary bacteriology and the communicable diseases.

Parts I and II will be conducted as written examinations in Class A Medical Schools and Part III will be entirely practical and clinical. In order to facilitate the carrying out of Part III, subsidiary boards will be appointed in the following cities, Boston, New York, Philadelphia, Minneapolis, Iowa City, San Francisco, Denver, New Orleans, Baltimore, Galveston, Cleveland, St. Louis, Chicago, Washington, D. C., and Nashville and these boards will function under the direction of the National Board. The fee of \$25.00 for the first part, \$25.00 for the second part and \$50.00 for the third part will be charged. In order to help the Board the Carnegie Foundation has appropriated \$100,000.00 over a period of five years.

At the Annual Meeting held June 13th, of this year in Boston, the following officers were elected, M. W. Ireland, Surgeon General, President; J. S. Rodman, M.D., Secretary-Treasurer, E. S. Elwood, Managing Director.

Mr. Elwood will personally visit all Class A Schools during the college year to further explain the examination, etc., to those interested. Further information may be had from the Secretary-Treasurer, Medical Arts Building, Philadelphia.



# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, JANUARY, 1922

No. 4

## *ORIGINAL ARTICLES*

### **BASAL METABOLISM DETERMINATION AND ITS TECHNICAL DIFFICULTIES\***

BY HARRY M. JONES, PH.D., CHICAGO, ILL.

IN discussing the technical difficulties encountered in determining the metabolic rate, it is obviously impossible to enumerate the many possible sources of such errors inherent in the various methods and devices now in use for making metabolic rate determinations.

There are, however, a few basic principles common to all these methods and devices, a thorough understanding of which is absolutely essential to any degree of success in their manipulation and use. There is also at the present time a most urgent need for a better understanding of these principles, failure to observe which has led to more doubt in the minds of clinicians today, concerning the real value of metabolism studies in disease, than the sum of all the other confusing sources of variations combined.

Clinicians everywhere are asking the question: In what diseases other than thyroid and pituitary abnormalities may the basal metabolism test be useful? while equally great numbers of clinicians everywhere are failing to make thorough application of the test in these conditions, in which it is known to be the most useful diagnostic aid in their possession. This paper is, therefore, a plea to the clinician to use with better skill that which is already known to be genuinely useful.

In the process of determining the metabolic rate by this or that method, the final result is obviously and always the algebraic sum of the plus and minus errors from at least three widely different sources, namely: (1) the test subject, who though in normal health, may have a metabolic rate varying several per cent from his expected rate, and who may cooperate irregularly, or not at all, in the performance of the test; (2) the technician, who must execute many details and observations besides those pertaining directly to the manipulation of

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the instrument: and (3) the apparatus itself, which may introduce error, perhaps consistent, perhaps variable, because of certain of its mechanical defects unknown to the operator.

If the errors of various technicians making metabolic rate determinations were listed under these three headings according to who or what caused the error, it would be found, that (1) the test subject, (2) the technician, and (3) the apparatus, share quite unequally as causes of error. In this connection, it is one of the unexplainable features of the beginner's attitude of mind that when errors occur, he invariably declares that his apparatus is not accurate. The responsibility for all errors, regardless of their real source, is invariably laid to the fault of the apparatus. As a matter of fact, the apparatus is hardly ever the cause of error.

If the final result of a given test proves to be obviously incorrect, as the result, for example, of an air-leak in the apparatus, it is the fault of the technician for not having examined the apparatus for air-leaks at the beginning and end of every test in accordance with one of its fundamental requirements. On the other hand, if the rate is found to be too irregular in "check" determinations on a given subject, as the result of the subject's failure to lie perfectly quiet during the test, it is the technician's responsibility to require the subject to conform to certain fundamental rules, or else refuse to make the test.

Furthermore, if the technician's failure to observe these fundamental requirements is due to his lack of understanding of these sources of errors, it is again his fault for not having learned these essentials before assuming such responsibility.

In the Spring of 1920, the author had devised and perfected a portable device for measuring the rate of metabolism. This apparatus, in its separate parts, represented such wide departure from those of other devices then in use for this same purpose, that it was considered desirable, if not essential, to make comparison of its results with those of other devices then in use in the various nearby hospitals and laboratories. It was in this connection that the present writer was obliged to depend on the knowledge and technical skill of the various operators of these instruments with which the comparisons were to be made.

Of the six comparisons made, apparatus No. 1 showed an unmistakably low reading of -17% on the normal test subject, due, it was finally discovered to a previous exhaustion of the  $\text{CO}_2$  absorbent; apparatus No. 2 gave a +21% reading on the same normal subject, due, it was found after an entire morning's delay, to a leakage of air from a large rubber-hose connection; apparatus No. 3 gave a -12% reading, due it was discovered after three days of "tinkering" with the apparatus itself, to an incorrect factor which had up to this time been used in the calculations of the gas volumes; apparatus No. 4 showed so many defects that the experiment was abandoned after three readings showing +72, +90, and +20 per cent. A fifth attempt was made by returning, then, to apparatus No. 1. This time the comparison was fairly successful, showing only a 7 per cent difference in the readings of the two instruments, but even here the reading from apparatus No. 1 was again incorrect because the technician had neglected to interpolate to the second decimal place for the body area, which

she admitted had been her regular custom. A sixth attempt was then made with apparatus No. 4, but a wide disagreement was found again because the technician according to his regular custom, stopped the electric motor immediately after disconnecting the subject from the breathing circuit, which obviously left a considerable volume of unabsorbed  $\text{CO}_2$  in the spirometer and breathing circuit.

Lest it be thought that this was a most unusual series of mishaps in making these comparisons, I feel impelled to admit that subsequently it has been my experience to find this deplorable state of affairs throughout all parts of the country. And any technician or clinician who feels that his own results are an exception to this, should see to his own technic in securing *normal readings on known normal subjects* before offering challenge to the proposition.

Usually if the reading on a suspected hyperthyroid subject falls by chance on the plus side of that patient's normal rate, the clinician accepts the result without question, because "it checks up clinically." That the subject may not be suffering from hyperthyroidism at all, or that the plus reading may be more or less "plus" than the rate really is, does not matter to him, so long as the results "check up clinically." With some, the source of error is from the calculations; with others it is from misreading the stop watch, or lack of a stop-watch, or the barometer, or lack of a barometer; with still others it is lack of information concerning the patient's preparation, and so on.

When told of the unsatisfactory results of other comparative tests, each technician, in turn, invariably hastens to assure one of the wonderful accuracy of his instrument. Others may be in doubt about their instruments, but he knows his apparatus is accurate. Does he make tests on subjects known to be normal? Why certainly not—normal subjects are not interesting. Does he run duplicate tests on his patients? No, duplicate tests would only give the same result, and besides they take too much time. Then, how does he know that his apparatus is accurate? Simply because the results always "check up clinically!"

This reassuring conversation is almost invariably followed by the usual disappointing result, namely, duplicate readings on a normal subject which are ridiculously at variance with each other, and which also disagree with what is known to be the normal rate. His failure this time to get his tests to "check up clinically" is registered as a definite depression in his enthusiasm, but not to be outdone, he still assures one that this is the first time the apparatus has been out of order, laying the blame again, in characteristic fashion, on the unoffending apparatus.

Unfortunately too many technicians merely skim through the reading of instructions and therefore have only a very superficial grasp of the underlying principles of apparatus and methods committed to their care. Only after a technician has had much experience in metabolism studies, will he appreciate what a really exacting task it is, this measuring the rate of metabolism. Any device used for this purpose, however simple and mechanically perfect, requires careful watching, as does any other exacting quantitative test, and the technician who gets into difficulty in making such a test is invariably one who has read his instructions hurriedly, and understands them superficially, or not at all.

Therefore, it is absolutely essential that the technician take time to read instructions carefully, and to read them through to the end before attempting to perform the test. Obviously each important point cannot be emphasized separately because every step in the technic is important, and no matter how perfectly the technician performs most of the test, the conclusiveness of the final result is no stronger than the weakest point in the rest of his technic since these are also links in the same chain, which produced that final result.

One should study the apparatus itself, in connection with the instructions, understand the purpose and working of each part, and follow out the reasons and principles involved in the whole procedure. They are merely elementary facts of science, long ago familiar to the technician, and their relations are so perfectly obvious when once connected as logical means to our aim, namely, to measure the rate of oxygen consumption. Only in this way will the clinician be in a position to interpret unexpected variations in the results, and to disclose and eliminate those hidden causes of error which are invariably encountered in any quantitative measurement.

Since (1) the test subject, (2) the technician, and (3) the apparatus, are severally responsible for the errors encountered in these tests, it is only common sense for the beginner to start with one of these factors as a known and relatively fixed quantity, namely, a test-subject in normal health, who will cooperate in maintaining for him at least relatively uniform basal metabolism conditions for practicing the test. In this way the other two sources of error may then be reduced to known quantities, and, in turn, eliminated.

Most every technician feels that, at least for him, this preliminary training is an unnecessary waste of time. After the first few attempts with the test one will readily understand why this try-out method is recommended for the beginner. On over one hundred different occasions the present writer has witnessed the beginning technic of over one hundred clinicians or their technicians. Out of this number, not one of them, during their first attempts, came anywhere near the proper handling of the various types of apparatus used for this purpose, and showed even less knowledge as to how the patient should be managed during the test. The test itself is usually so simple, and some of the foolish blunderings of otherwise intelligent technicians are so inexcusable, and their mistakes in diagnosis of such far-reaching consequence, that it has been a source of constant wonder that suits for malpractice are not the rule rather than the exception through the abuse of this most valuable diagnostic aid.

Another peculiar feature of the beginner's attitude of mind with reference to apparatus used for determining the metabolic rate, is that invariably, when left to follow his own desires, he turns at once to pathologic cases to "try out the apparatus." Such an "accuracy test" would be even more illogical than testing the accuracy of a clinical thermometer on a patient with an unknown rise in temperature.

Practically every error in technic which the beginner is likely to make is such as would lead to unusually wide variations in the final reading of the test and therefore to gross error in diagnosis. Thus, in spite of repeated warning, one favorite blunder of the novice is to read the time, e.g., 2 minutes and 55 sec-



onds, from the stop watch as 3 minutes and 55 seconds. Warning him of this is useless. He thinks he should at least know how to read a stop-watch.

The reason for the frequency of this error is that the minute-hand of the watch stands so near to the succeeding minute mark when the second-hand is approaching the last few seconds of the preceding minute. To ask a technician to avoid this particular error is an obvious reflection on his intelligence. When, in spite of warning and explanation, however, he finally is caught in the error, he usually flushes up a bit and passes it off, as though it amounted to nothing anyway, not knowing that it amounts to about a 25 per cent error in the final calculation of the metabolic rate.

For example, on one occasion the technician reported a +8 per cent reading, on a suspected hyperthyroid patient. This rate being within the normal limits, the patient was dismissed as a "neuro." The physician four months later became convinced that some error in the test had been made, and ordered the technician to make a second test. This time, however, the technician who had meanwhile learned to avoid the above source of error, found the rate to be +58 per cent. This unexpected increase and the record sheet of the previous test led to the discovery that, because of a reading of 3 minutes and 52 seconds, the rate as determined two months ago should have been reported as +35 per cent instead of +8 per cent. Now, however, the patient was so much worse, that radical operation, although advised against, when performed two weeks later, eventuated fatally. One can say nothing less than that failure to observe a specified point in the technic had allowed the golden moment for successful operation to slip by.

On another occasion, a clinician was demonstrating the metabolism test to a group of visiting clinicians. He had never made the test before, and the apparatus to be used for this purpose had just been received. In characteristic fashion, he had turned at once to pathologic conditions, and had gone to much trouble to have a hyperthyroid patient serve as his subject. The demonstration had been a failure after several attempts, and it was at this point that the clinician called me by telephone to ask how so and so's method was supposed to affect the patient.

Half an hour later, when I had joined the other visitors, the test was tried once more. When the test began, the patient was comfortable, breathing normally for the first 30 or 40 seconds, but after about one minute, the patient drew up her knees, rolled the eyes upward, changed to a deep scnorous type of respiration, the face became deeply cyanotic, the jaw relaxed, and fortunately for her, allowed the mouth-piece to drop from the mouth.

Gas anesthesia!

Not by the wildest flight of imagination could the investigator, who designed this new method, have conjured up the possibility of so huge a blunder, or anticipated a warning to those who used it, not to give gas anesthesia when doing metabolism determinations. A tank of gas, supposedly oxygen, but labeled "Nitrous Oxide" stood nearby as mute witness to the clinician's "oversight."

Many other equally unfortunate instances could be cited to show how absolutely essential it is to *have the technician practice with known normal persons,*

whose metabolic rates are known, within reasonably narrow limits, and whose management during the test is relatively simple as compared with the tactful and varied procedures necessary with the highly excitable hyperthyroid patient or the temperamental neurotic. To demonstrate his ability to make a reading sufficiently dependable for clinical use, it is only common sense to require that he begin with at least one known quantity, namely, test cases of known metabolic rate.

One should not imagine, however, that the values given in the table of so-called normal rates, are absolute values. A normal person may vary above or below these values (commonly 10 per cent, rarely 20 per cent) and still be regarded as in good health. These rates should be called averages rather than normals. Most of the readings, however, if properly made, will come within a few per cent of these values, but one should not, as some technicians do, become discouraged if the first normal subject tested should chance to be one of those rare cases whose rate varies regularly several per cent above or below the expected rate. One should simply continue the tests on still other normal persons, until the cause of uncertainty is discovered.

Moreover, when the technic is finally mastered, and the technician has become thoroughly acquainted with the features which require constant watching, he should, in the midst of his many tests on pathologic subjects, frequently return to known normal subjects to reassure himself that some undetected mechanical defect in the instrument has not developed recently, and thus introduced an unexpected source of error. These insidious sources of inaccuracy are far more numerous than commonly known, and the only method by which they may be detected, and which is always available to the technician, is to return again to fundamentals and require the instrument to give normal readings on known normal persons.

That the skill and knowledge of the technician must be adequate to the task of producing a normal reading on a normal subject is so obvious as to require no argument, but the difficulty seems to be that this amount of skill is taken for granted, and the clinician wants a short cut to direct results with his cases showing real pathology.

Another limitation of the technician's skill no less important than his ability to secure normal readings on normal subjects, is his ability to secure uniform results in successive tests on the same subject under the same conditions. Is his technic so standardized that two or more determinations on the same subject tell the same story?

Here, again, such an obvious situation is taken for granted, namely, that the same causes must produce the same results, and time is too valuable to spend in useless, uninteresting repetition.

One clinician, for example, when asked how his readings on the same subject agree with each other, replied that he never made duplicate determinations since any one test was always accurate to at least one one-hundredth of one per cent, and that this was close enough for his purpose, without a duplicate test! This technician did not know that the average limits of accuracy of any method

devised so far, show at least 2 per cent variation in its own readings, and that the method used by him is the most variable of them all.

Then, on the other hand, the technician who has been told the limits of accuracy of such and such an instrument or method, takes it for granted that this accuracy is an inherent part of the device itself.

Devices may differ in the number of manipulations necessary to reach the end result, and, in this way, in the number of chances for possible errors through such manipulations, but they do not differ in their absolute dependence upon a thorough knowledge of the technic required for their operation.

Regardless of the method or device employed, the rate of metabolism is stated in terms of calories, these calories in turn being determined in terms of the amount of oxygen used by the subject. Therefore, *the brunt of the calculation falls entirely upon the accuracy with which the oxygen volume is measured.*

Now, since gases, which must be passed and repassed through tubes, spirometers, rotary-blowers, face-masks, mouth-pieces, gas-cocks, and what not are notoriously subject to rapid losses through the most minute points of escape, the amount of escape being the amount of error in calories, the latter may very quickly become considerable.

Since these points of escape may develop at any time between tests, or even during the test, a single determination may be seriously in error without obvious cause unless the gas-tightness of the apparatus be proved before and after the test, as a routine part of the technic. With certain forms of apparatus a leak will not develop after the apparatus is once closed ready for the test proper, but with other forms this mishap is common, and it is clearly the technician's responsibility to be constantly on guard against such unexpected sources of error.

By using the duplicate test as a regular part of the routine, these and other irregular causes of error, such as face-mask or mouth-piece leaks, errors in calculation, misreading the stop-watch, and so on, are also detected, and many an embarrassment in diagnosis spared the clinician.

#### SUMMARY

1. The technic of the operator must be proved adequate to the task of securing normal readings on known normal subjects. One need not quibble about this. It may be difficult to secure the service of a normal person for a subject, it may take much of one's valuable time to make these uninteresting tests, but *the clinician positively cannot rely on his results with suspected pathologic cases until this fundamental principle has been observed.* The reason this principle needs such repetition and emphasis is that every clinician, without exception, imagines that the apparatus is accurate if it will give a plus reading on a known hyperthyroid patient. It does not occur to him that such a test is absolutely no proof of accuracy, since the apparatus may be very inaccurate and still give the desired plus reading. In other words, the reading may be any amount of per cent plus and the clinician still be in no position to dispute with the instrument whether or not this is the correct plus percentage.

2. Having learned the technic on normal subjects the tests may subsequently, in the routine of business, become a long sequence of determinations on only pathological cases, during which time some unknown mechanical defect may have introduced an undetected source of error. This requires that the clinician return to fundamentals again, and eliminate the possibility of these sources of error by securing normal readings again on subjects known to be normal, *not depending for this on occasional subjects sent in as patients which may or may not be normal.*

3. If any quantitative determination is unsafe without a duplicate determination, or appropriate controls, which serve the same purpose, basal metabolism determinations are doubly unsafe, because of the great complexity of details in the technic and the far-reaching consequence of their errors. Common sense will sanction the statement that this applies to any device or method used for this purpose, regardless of who or what institution vouches for its accuracy, and regardless of the amount of time required for duplicate tests. A method which requires so much time for one test as to make the two tests impracticable, is unfortunate in requiring so much time, but is not thereby made an exception to the fundamental rule that *any method or device, to be dependable, requires a duplicate test for a control.*

Recently a physician asked to have two check determinations made on himself, by what has been widely reported as the most accurate method now in use, and by a technician who had made determinations then numbering into the hundreds, but who had always omitted the check test for lack of time, "for such unnecessary labor." The two readings were -21 per cent and -7 per cent, respectively. Somewhat embarrassed by this disagreement, the technician agreed to make two more determinations on the following day. This time the readings were +4 per cent and -4 per cent, respectively. This physician was so genuinely disappointed with the results that his honest cooperation during the tests cannot be questioned. Furthermore, he subsequently went over the results of the gas analysis and calculations with the technician to assure himself that the wide variations were not from these sources. According to report, such wide variations on the same subject do not occur, but in practice they do, and in the hands of the unskilled, they are even greater than these. When they will occur, can never be known beforehand. Why they occur, is another story.



# THE INFLUENCE ON SPORE FORMATION OF SEALING BACTERIAL CULTURES\*

BY LAURA FLORENCE, PH.D.

DURING a study of the pathogenesis of *Bacillus abortus* Fabyan (1912), continuing the work of Smith and Fabyan (1912), stated that the chief problem in the cultivation of the organism was to obtain successful cultures from the tissues. The method followed was that of Nowak with slight modifications, but later it was found that by merely sealing the culture tube with sealing wax, and incubating it longer in some cases, satisfactory results were obtained. Since then the latter method has replaced all others in this laboratory in the cultivation of *Bacillus abortus*. Also, it has been the means of the discovery by Smith (1918, 1 and 2) of two new organisms, viz.: the *Vibrio fetus* of infectious abortion in cattle and *Bacillus actinoides* which may be representative of a hitherto unknown group of organisms capable of growth, at least at the start, only under such conditions. The question naturally followed, what is the general effect on culture growth of such a method? To discover this a series of experiments was begun during the winter of 1918-1919 and transfers of a number of stock cultures were grown in parallel series of sealed and unsealed cultures. The results showed the necessity of confining the observations to some well-defined biological process, and spore formation presented itself as the most suitable. The work was limited first to a study of some of the common sporebearers and finally to a more detailed study of the anthrax bacillus.

The organisms used in this investigation were *Bacillus cereus*, *Bacillus mesentericus fuscus*, *Bacillus mesentericus vulgaris* (2 strains.) *Bacillus Welchii*, *Bacillus anthracis*, and two unidentified bacilli, one isolated from the lung of a calf and designated "*Organism A*," and one isolated from the liver of a guinea pig and designated "*Organism B*." *Organism A* is in size and appearance not unlike *Bacillus mesentericus vulgaris*. It is actively motile and central spores appear within 24 hours. On solid and liquid media it grows as a wrinkled membrane. In bouillon a faint turbidity appears in 24 hours and gradually increases in density during the life of the culture. It grows on potato, liquefies gelatin, and slowly coagulates milk with an extrusion of whey on the 4th day. In the sugars there is no gas production and the reaction is moderately acid. "*Organism B*" is a slender rod with rounded ends. It has a moderate motility and forms small terminal spores. In deep agar shake cultures, small subcircular colonies with an irregular surface appear within 24 hours and, as they grow, they become rhizoid in form. Numerous gas bubbles are formed.

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Received for publication, July 7, 1921.

To determine the relation of these organisms to oxygen, cultures were grown in fermentation tubes of dextrose bouillon after the method of Smith. In 1893 he had explained the use of the fermentation tube for such purposes and two years later (1895)) emphasized the relation between sugar and anaërobiosis. The results of the test grouped the organisms as follows:

- |                          |  |
|--------------------------|--|
| 1. Obligatory aërobes    | { <i>Bacillus mesentericus fuscus</i><br><i>Bacillus mesentericus vulgatus</i> (2 strains) |
| 2. Facultative anaërobes | { <i>Bacillus anthracis</i><br><i>Bacillus cereus</i><br>Organism A                        |
| 3. Obligatory anaërobes  | { <i>Bacillus Welchii</i><br>Organism B.   |

A uniform method of closing the culture tubes was used. All agar slants were closed immediately after inoculation with paraffin-dipped cotton stoppers cut off level with the top of the tube and then pushed down approximately one-sixteenth of an inch below the top. The mouth of the tube was flamed until thoroughly hot, when a small amount of sealing wax was placed over the stopper. This was absorbed by the stopper and, when the tube had cooled, the space above the stopper was filled with sealing wax, care being taken to leave no air bubbles. Cultures grown in bouillon and on potato were closed in the same way as the agar slants except that the stoppers were not dipped in paraffin.

Under such conditions the development of an organism is affected by a number of factors. As already pointed out, such an environment favors the growth of some organisms. Moreover it had been stated by Pasteur (1880) that hermetically sealing the necks of his flasks preserved the virulence of his cultures of the organism of chicken cholera over long periods of time. In spite of the exclusion of the air two sources of oxygen may still be available to the organisms. There is first that enclosed in the tube when sealed, and second that which may be contained in the media. It was found by Hesse (1893) that, during the growth of organisms in markedly alkaline media in air-tight tubes, a considerable quantity of the available oxygen was absorbed by the media, but in media of such moderate alkalinity as is usual in routine work the quantity of oxygen so absorbed was negligible. However, that a reduction in the oxygen tension does gradually take place in sealed tubes of media of a moderate alkalinity can be proved by adding a few drops of a sterile aqueous solution of methylene blue to the tubes at the time of sealing. Reduction phenomena coincident with bacterial growth have long been known, but Spina (1887), alone of the earlier workers, has described its occurrence in sterile media—bouillon and gelatin—in hermetically sealed tubes. Some nine years later Smith (1896), while using the fermentation tube in his study of the reducing action of bacteria, also found that sterile culture media exhibit reducing properties. In these two cases the reduction takes place in what may be described as an air-free environment with consequent rapid loss of color. If, on the other hand, the colored medium is contained in ordinary culture tubes closed with sealing wax, there will elapse, before the color change takes place, a variable period of time.

The experiment was carried out with bouillon and agar; each tube contained 6 c.c. of medium and the agar was slanted. The medium was placed in a water-bath and after reaching boiling point was allowed to boil from 10 to 15 minutes. It was cooled quickly to 45 to 47°C. and definite quantities of a 0.1 per cent sterile aqueous solution of methylene blue added. One-half of the tubes were cooled before sealing and the remainder were sealed immediately on taking from the water-bath. The tops of the latter were thoroughly heated in the flame, a tightly fitting circle of sheet asbestos was placed over the cotton, and they were then sealed as quickly as possible. The asbestos prevented the sealed plug from being drawn into the tube as it cooled. The results given in Table I show not only that the decoloration took place slowly but also that the time taken was not uniform in the different tubes.

TABLE I

MEDIA	QUANTITY OF 0.1 PER CENT METHYLENE BLUE	NO. OF DAYS FOR COMPLETE DECOLORATION IN INCUBATOR		NO. OF DAYS FOR COMPLETE DECOLORATION AT ROOM TEMPERATURE	
		SEALED HOT	SEALED COLD	SEALED HOT	SEALED COLD
Agar	C.C.				
	$\frac{1}{2}$	37	40	33	—
		40		30	33
		69	69		
	$\frac{1}{4}$	18	—	22	33
		17	—	20	—
	$\frac{1}{6}$	12	54	17	35
	$\frac{1}{4}$	13	20	17	20
Bouillon	$\frac{1}{2}$	64	—	—	33
	$\frac{1}{4}$	14	—	—	26
	$\frac{1}{6}$	—	13	19	—

Tubes sealed hot become quite decolorized a little more rapidly than those allowed to cool before being sealed. The rate of the reduction was also moderately influenced by the temperature at which the tubes were kept, and was to some extent proportional to the quantity of coloring matter introduced.

In the study of the common spore-bearers, bouillon and agar were the media used. If transfers were made from cultures containing spores, the majority of the spores carried over did not germinate in the sealed tubes, so it was necessary to make the transfers from cultures free of spores. To obtain these two methods were tried: first, making transfers from cultures grown 6 to 8 hours, and second, heating freshly inoculated cultures containing spores above the thermal death point of the vegetative forms and incubating them for a number of hours before sealing to allow the surviving spores to germinate. The former procedure was the more satisfactory and was used almost entirely throughout the experiments.

#### EFFECT OF SEALING ON MACROSCOPIC GROWTH

In order to determine the effect of sealing on macroscopic growth the aérobés and facultative anaérobés, with the exception of the anthrax bacillus, were first grown in bouillon cultures, when the following results were obtained:

## UNSEALED CULTURES

All organisms grew well.

## SEALED CULTURES

The aërobes showed a very moderate growth in 24 hours which had not visibly increased at 48 hours. After 5 days the culture of *B. mesentericus fuscus* appeared like sterile bouillon, while the others showed traces of a thin surface membrane which later disappeared.

The facultative anaërobes grew as well as in unsealed tubes for 48 hours, when macroscopic growth ended. After 9 days the growth began to disappear and after 23 days only a small amount of sediment remained to distinguish the cultures from sterile bouillon.

In films made from sealed bouillon cultures incubated 37 days without having been unsealed, few organisms were found, vegetative forms and spores being present in approximately equal numbers. When the cultures were unsealed and incubated, growth appeared in all before 48 hours. In films made from sealed agar cultures incubated 75 days without having been unsealed, vegetative forms and spores were again found in approximately equal numbers. When the agar cultures were sealed and incubated, growth appeared in all after 3 days.

## EFFECT OF SEALING ON SPORE FORMATION IN AEROBES AND FACULTATIVE ANAEROBES

In determining the time of appearance of spores parallel sets of cultures on agar were grown in all cases, some of which were examined daily, and others at intervals of 2, 4, and 6 days. Of each of the aërobes and facultative anaërobes a series of thirty to forty inoculations was made at one time, to be opened on successive days, but the results did not differ materially from those got from cultures repeatedly opened for examination and resealed. By series of cultures is to be understood all the inoculations of an organism made at one time. A study of these series showed the following results:

## UNSEALED CULTURES

All organisms grew well producing spores within 24 hours.

## SEALED CULTURES

In the first series of aërobes spores appeared after 27 to 30 days' incubation, and in a second series, inoculated from the first, after 32 to 36 days' incubation.

In the first series of facultative anaërobes, spores appeared after 25 to 35 days' incubation in "*Organism A*," and after 19 to 26 days' incubation in *Bacillus cereus*. In a second and successive series to the sixth, spores appeared after 30 to 35 days' incubation.

In rate of growth and cessation of growth agar cultures resembled bouillon, but on the former the organisms died more slowly. When spores first became visible they were present in very small numbers and in most cases were still within the vegetative forms. The change from vegetative forms to spores took place very gradually and in cultures that had been incubated 60 to 75 days, spores within vegetative forms were found. Some of these organisms may have died in that condition, but such cultures, if unsealed and incubated, showed new growth in 2 to 3 days.

Parallel series grown in tubes sealed 1, 2 and 3 weeks before inoculation and kept in the refrigerator, at room temperature, and in the incubator, gave results corresponding to those obtained from cultures grown in tubes sealed first at the time of inoculation. This might not be the case if it were possible to inoculate the tubes without exposing them to the atmosphere.



Parallel series grown at room temperature and in the incubator did not differ appreciably in growth or in the results obtained.

After five days for the aërobes and 9 days for the facultative anaërobes the number of the organisms in hanging drops and films began to diminish. That the majority died without forming spores was suggested by the many nonmotile forms present in hanging drops and the many involution forms in films.

#### EFFECT OF SEALING ON SPORE FORMATION IN OBLIGATORY ANAEROBES

In the case of the two obligatory anaërobes no retarding of spore formation occurred, and the presence of spores at all stages of growth was confirmed by means of heat tests. Certain facts seemed to indicate, however, that the conditions under investigation were not highly favorable to these organisms. In agar slants, to the condensation water of which small pieces of sterile guinea pig tissue had been added, no better growth was obtained in sealed than in unsealed tubes. In both cases the growth appeared rather slowly and a number of such transfers were negative. Transfers made to agar slants without tissue and sealed rarely showed growth and, if left unsealed, no growth appeared. In these some of the vegative forms carried over at inoculation may have formed spores, but in many cases they appeared to die without undergoing any change, as they were found in films and hanging drops of cultures which gave negative results, when transfers were made from them to agar shake cultures.

*Bacillus Welchii* grew somewhat more readily than *Organism B*, growth appearing within 24 hours in the former and usually within 48 hours in the latter but in a few cases not for several days. The earliest indication of activity of the organisms was the formation of gas between the curved surface of the agar slant and the glass. The bubble increased in size generally for a period of 2 days, when the gas escaped wholly or in part from behind the agar. The formation of gas was followed by a slow growth of the organism between the medium and the glass. *Organism B* grew in a thin diffuse film and *Bacillus Welchii* in well-defined colonies. In sealed agar slants containing guinea pig tissue the organisms retained their vitality for a considerable time, and transfers to deep agar tubes from cultures which had been incubated upwards of 150 days gave positive results. Films stained with methylene blue and hanging drops showed vegetative forms in cultures 144 days old. The presence of numbers of dead vegetative forms in old cultures would indicate that the conditions were not altogether suitable for spore formation, though a limited number of spores were always present.

#### THE ANTHRAX BACILLUS

The results obtained when the anthrax bacillus was grown in sealed culture tubes proved to be so variable that a number of experiments were carried on with this organism alone. Two strains were used but no difference was detected in their reaction to the conditions of growth. A number of publications dealing with spore formation in anthrax appeared at the beginning of the present century. These will not be discussed here, because the writers

were experimenting with conditions of complete anaërobiosis and moreover, they have already been reviewed by Sobernheim (1913).

In a series of eight transfers of the anthrax bacillus made from a spore-free culture and grown on sealed agar slants, spores were not found in seven cases until after the 27th day. The examination of films and hanging drops of the cultures showed that the majority of the vegetative forms did not retain their normal outline as was the case with the other spore-bearers studied, but gradually came to resemble single cocci and chains of streptococci. This experiment was repeated four times and in no case were the definite results of the first experiment obtained.

The organism was grown in sealed culture tubes and flasks of different sizes to discover the bearing of the quantity of enclosed atmospheric air on the time of appearance of spores. Culture tubes of the usual diameter but of three different lengths, viz. 12 cm., 15.5 to 16 cm., and 20.2 cm., were used. No appreciable difference was found between the results obtained with the first and second of these, but the time was considerably shortened when the third length was used. In the former, spores were first found after 27 to 36 days but in two cases none were found after 64 days, while in all the latter, spores appeared at the 10th day. That the organisms in the 64-day culture were still alive was proved by the growth of a transfer made from it before the heat test for spores was made. Cultures were also grown on agar in Erlenmeyer flasks of 100 c.c. and 250 c.c. volume and in Blake bottles of 500 c.c. volume. In the two last, spores regularly appeared within 24 hours and in the first within 48 hours. Bouillon was substituted for agar in the 100 c.c. flasks, 10 c.c., 20 c.c., and 30 c.c. being put in each of three flasks, and in every case spores formed during the first 24 hours. These results showed that as the ratio between the surface of the medium and the volume of the air enclosed was increased, the time before the appearance of spores was decreased.

Since the anthrax bacillus grows well on potato and as a rule numerous spores are produced while the culture is still young, tubes of potato similar in size to the usual agar slant tubes were prepared in three different ways and two varieties of potato used. Sobernheim (1913) considered the variable chemical composition of potato, especially its reaction, to influence not only the growth of the organism but also spore formation. Five series of cultures were grown on potato. In the first three series the potato was prepared according to the usual laboratory method and washed for 2 hours in running water, in the fourth series three potatoes of the same variety were used and were washed in running water for less than 1 hour, and in the fifth series potatoes of two different varieties were prepared also with short washing. In every sealed culture of all five series spores were present at the end of 24 hours and were almost as numerous as in the parallel series of unsealed cultures. Growth in the unsealed tubes was characterized by the formation of a brownish pigment, while in the sealed tubes it was always a smooth glistening white. Special potato media were next prepared. A potato was peeled and cut up, boiled to a pulp in distilled water, strained through muslin and 1 per cent agar added to the extract (a). From others (b) the starch

was separated, and extracts made containing (e) the salts with the protein and (d) the salts without the coagulable protein. These preparations were each made up with 1.5 per cent agar. Parallel series of sealed and unsealed cultures were made on all these media. The organism grew well on the potato extract agar (a) forming a brown pigment in the unsealed tubes; it grew moderately well on the salts with protein extract agar (c), and on the salts extract agar (d), but it did not grow on the starch extract agar (b). The results coincided with those obtained from the usual potato cultures, and in all tubes, sealed and unsealed, in which growth occurred, spores formed within 24 hours after inoculation.

In order to discover whether the reaction of the media influenced spore formation in sealed cultures, acetic acid was added to three series of bouillon tubes whose resulting titratable acidity varied from 0.45 per cent to 2.45 per cent and pH from 7.8 to 5.8. Also to six series of agar slants normal sodium carbonate or acetic acid was added to produce titratable acidities varying from 0.1 per cent to 2.8 per cent. In both experiments the results were variable and showed that some important factor influencing spore formation had not been found. Growth on beef extract agar with titratable acidities corresponding approximately with those of the above agar series threw no further light on the point under investigation, but it was of interest that neither in unsealed nor sealed tubes having the two highest titratable acidities would the organism grow. The explanation was found to be lack of buffer substance and, when the buffer value of the media was increased by the addition of phosphates without changing the hydrogen-ion concentration, pH 6.8, the organisms grew equally well in the whole series.

When sugar-free fermented bouillon and agar were used, spores regularly appeared within 24 hours after inoculation in both sealed and unsealed cultures. The addition of a 0.1 per cent or a 0.2 per cent solution of dextrose to two parts of the bouillon slightly accelerated the rate of spore production.

The fact that on the special media prepared from potato the organism had grown moderately well on the salts extract agar (d) suggested that salts might be an important factor. 1 c.c. of  $\frac{M}{5}$  solutions of the potassium phosphates were added to 6 c.c. portions of agar and the medium sterilized and slanted. In all cultures spores developed within 24 hours. The sodium phosphates were used in two experiments along with three different media. In the first experiment a constant pH 7.4 was maintained and 1 c.c. portions of seven different concentrations of the salt were added to 6 c.c. of media, while in the second experiment one salt concentration,  $\frac{M}{2}$  was added to give five different hydrogen-ion concentrations, viz., pH 4 to pH 8. The three types of media used were (1) plain beef agar, (2) 1.5 per cent agar in distilled water, and (3) the salts extract of potato (d). All the cultures grew well on (1) showing that the salt had no toxic effect in the concentration used and numerous spores were produced in less than 24 hours. On the second medium (2) no growth occurred. In the presence of the salts from potato, medium (3), spores ap-

peared in both sealed and unsealed tubes within 24 hours, and the best growth was obtained on the media having a hydrogen-ion concentration of from pH 6 to pH 8. Poor growth was obtained on media with a hydrogen-ion concentration of pH 4 or to which a very low concentration of the phosphates had been added. In the latter case the organism seemed to grow slightly better in the sealed than in the unsealed tubes.

To potato extract medium (d) were also added (1) a 1 per cent solution of casein aminoids, (2) a 1 per cent solution of asparagin, and (3) an 0.2 per cent solution of dextrose. These solutions used alone or in a combination of casein and sugar and asparagin and sugar did not favor the growth of the organism, and spores were formed in less than 24 hours in all cultures. The addition of casein along with the sodium phosphates to the potato extract agar (d) influenced neither the growth of the organism nor the rate of spore production.

In the course of the above experiments repeated comparisons were made of films and hanging drops prepared from sealed cultures grown on ordinary agar with those prepared from sealed cultures grown on media to which salts had been added. The presence of salts was found to increase the rate of spore production and, since both potassium and sodium salts produced the same effect, it may be the regulation of the reaction by the salts which is an essential factor in controlling spore formation under partial oxygen tension.

#### MORPHOLOGY

The exclusion of a free supply of atmospheric air brought about a marked change in the morphology of the anthrax bacillus, while in the other forms studied, very little if any change appeared in the vegetative forms. The process of spore formation in anthrax was studied by Ruzicka (1910) and he used a special medium which retarded the growth of the organism. This condition affected the vitality of the bacilli and the first steps of the changes which he described are identical with those seen in cultures grown in sealed tubes. According to Ruzicka the contents of the individual organisms lost their homogeneous appearance and became markedly granular, while some of the granules reached a considerable size and appeared black and refractive, not unlike small pores. Similar forms were later occasionally seen by Sobernheim (1913) in unsealed cultures grown on potato. In sealed cultures we have usually found them in considerable numbers after 3 to 4 days' incubation and in hanging drops they resembled cocci. The protoplasm surrounding the granules disappeared leaving only the refractive parts and before these became quite detached from one another they resembled short chains of streptococci. That these were not ripe spores was proved by the facts that they stained readily with methylene blue and were not heat resistant. *Bacillus anthracis* is Gram positive, but these forms were Gram negative. Since their formation was identical with the first steps in spore formation as described by Ruzicka, they were probably spores whose complete development was prevented by lack of sufficient oxygen and might be called spore-like bodies. In hanging drops made from cultures which had been sealed for a number



of weeks without being opened, almost all the organisms were of this coccoid form and, if such cultures were left unsealed and incubated, in less than 24 hours these had reverted to normal vegetative forms. Also, in tubes which were being repeatedly opened for examination these spore-like bodies were less numerous, even in the oldest cultures, than in tubes which were seldom unsealed. In hanging drops they were at times seen clumped together in zoögleic forms and at times they showed an active Brownian movement. The former had been noted as characteristic of anthrax bacilli in the absence of oxygen by Wood in 1889. Attempts were made to transfer these spore-like bodies to agar slants which had been sealed when the original culture was made and incubated along with it, but their form was not constant and they gave rise always to typical vegetative forms. They cannot then be considered mutants such as Henri (1914) obtained by exposing cultures of anthrax to the ultraviolet ray.

#### SUMMARY

The reduction of methylene blue in culture tubes of sterile media closed with sealing wax proves that a reduction in oxygen tension can be obtained by this simple method. The moisture of the medium is also preserved over a long period.

Sealing cultures retarded the formation of spores and killed the majority of the vegetative forms of the aërobes and facultative anaërobes but not of the obligatory anaërobes. It affected the vegetative growth of obligatory aërobes more rapidly and intensely than that of facultative anaërobes. It did not apparently affect the growth of obligatory anaërobes as compared with that in unsealed culture tubes containing a small piece of sterile tissue.

Among the aërobes and facultative anaërobes spore formation was retarded a few days longer in the second sealed series inoculated from the first than in the first sealed series, after which no variation in the time of appearance of spores occurred in cultures carried through six successive series.

The results obtained with the anthrax bacillus differed from those of the other facultative anaërobes and showed no uniformity. Experiments with different salts appeared to increase the rate of spore production and the regulation of the reaction by the salts may be an important factor controlling spore formation under partial oxygen tension. The absence of a free supply of oxygen caused a temporary change in the form of the bacillus.

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## SOME STUDIES ON THE ELIMINATION OF MOLDS FROM BUTTER\*

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THE following paper is a summary of the work done for a large butter-making plant which was troubled with their butter going "off grade" during the interval between the time it was shipped and the time it was consumed. This was happening so frequently and the money loss was so annoying that it was decided to call on the bacteriologist for assistance with the problem. Before taking up the study it was thought best to look up what previous published work was available along these lines and it was surprising how little could be found on so important a matter. This was probably due to the fact that dairy publications and Agricultural Experiment Station bulletins are not very widely circulated outside of those particularly interested.

The presence of molds or yeasts was what this study was undertaken for primarily, so literature on this subject was especially consulted. It was found that this subject had been discussed at the 16th Annual Meeting of the Wisconsin Butter Makers' Association<sup>1</sup> and that W. B. Combs and C. H. Eckles<sup>2</sup> had also done considerable work upon it.

In none of the literature available was a technique given for such work as we were to undertake, so a method had to be developed. While engaged in this, a study was made of the operation of the plant; here it was found that the raw cream from many farms and of varying ages was all poured into one large vat, steam being used to remove the last of the cream from the cans. From this vat the cream was pumped into the forewarmer where it is heated to between 90° and 100° F. and its acidity tested. While in this stage of the process sufficient milk of magnesia suspension is added to bring the total acidity down to between 0.35 per cent and 0.4 per cent. After thorough mixing the cream runs by gravity to the pasteurizer, where its temperature is raised to 148° F. The cream takes one minute to pass through this step in the process, finally reaching the holding vat, which it enters through a strainer, and it remains here twenty minutes, a series of baffles insuring thorough mixing and uniform exposure to the heat.

From here at a temperature of 142° F. the cream is elevated to the top of the cooling coils, through which cold water is pumped, and after flowing over these enters either the ripener vats or the churn at a temperature of 65° F. The cooling coils are not enclosed, neither is the holding vat covered. Mention has been made of the "ripeners" vats. These are large tin-lined boxes with a capacity of 1,000 gallons of cream in which, if "starter" is used, the lactic acid culture is added and the whole mixed well and allowed to stand at a temperature of 52° F. for two hours in order that the lactic acid

\*From the North Dakota Public Health Laboratories, Grand Forks, North Dakota.

bacteria may multiply and raise the acidity in order that the bacteria which may have missed death in pasteurizing will be destroyed and the cream will also churn better.

"Starter" is the name given to the lactic acid culture which is prepared fresh each week from a pure culture received from a dairy supply house. The method of preparation is the familiar one used in homes where pancake batter is always kept in a jar behind the stove. A little of the active principle is added to a batch of pasteurized sweet milk, in this case a pure culture of lactic acid bacilli, allowed to grow overnight at room temperature and the resulting mixture is run into ten gallon cans and used as a "starter" for the big tanks described above.

To return to our cream. As has been said after leaving the cooler it may go to the ripener or to the churn. The cream is churned in large rotary churns for one hour, salt and coloring matter being added during the process, salt and moisture determinations being made before the product is finished. The butter is removed from the churns by hand and packed in tubs or pound cartons being then ready for sale.

A study of the equipment showed that there was no cover to the holding vat and that the cooler was open on all sides, flies and dust having free access. In busy times when the plant was run to capacity, 30,000 pounds of butter were produced, two shifts being employed. It was found also that the employees were quite likely to neglect to thoroughly clean the complete system and that leaks in the pipes allowed dirty water in and cream to run out. The ceiling of the main work room was of typical factory construction being actually the floor and supporting joists of the floor above which had been white-washed at one time, but was now spotted with what proved on examination to be, a growth of black mold.

After some consideration of the matter, it was decided to make bacterial counts on the cream at the various stages in the butter making process and at the same time make cultures for the presence of molds. Plain agar made according to the directions contained in the A. P. H. A. Standard Methods for the Examination of Milk was used for the plate count and a 1 per cent maltose agar was used for the fungi. The agar plates were counted after 48 hours in the incubator at 38° C. and the maltose plates were examined after four days in a dark drawer at laboratory temperature.

Samples were taken from the cream, entering the pasteurizer, entering the holding vat, leaving the holding vat, bottom of the cooling coils, ripener, entering the churn, buttermilk, butter. Before proceeding with the results of these tests in detail, it is interesting to note that the commercial value of butter is determined largely by its flavor and aroma. These two items are due to the by-products formed by the fermentation of the milk and cream and held in combination with the fat. If, therefore, the bacteria and other organisms present produce a disagreeable result, the keeping qualities of the butter and its flavor and aroma will be affected also. It is thus manifestly the duty of the buttermaker to so control his product that only the desirable bacteria shall predominate.

It was found in preliminary tests that the cream was handled so carelessly by the ordinary farmer that it was necessary to make dilutions of 1-10,000 in making the plate counts. The following table is typical of many of the results obtained for the first few months.

TABLE I

NO.	SAMPLE COLLECTED FROM	DILUTED 10,000 TIMES 1 C.C.		TEMPER- ATURE F	ACID- ITY %
		AGAR	MALTOSE		
5	Entering pasteurizer	230,000 1 mold	2 white molds; 3 green center molds	157	0.40
6	Entering holding vat	60,000 1 mold	1 mold; green center	156	
7	Leaving holding vat	10,000	1 white mold; 2 green center molds	154	
8	Bottom cooling coils	40,000	1 white mold; 1 green center mold	81-82	
9	Entering ripener	20,000	5 green center molds; 4 white molds	64	
13	Entering churn	2,260,000	2 white molds; 2 green center molds		
14	Buttermilk	4,790,000	2 blue center molds; 5 white molds; 6 green center molds		
	Butter				

At the time this investigation was begun “starter” was being used. Examination of the “starter” as it was prepared for use in the ripener vats showed molds to be present, so that if through careful operation of the pasteurizer and maintenance of an even temperature in the holder and careful attention to the pipes and cooling coils, the cream was free from these when it arrived at the ripening vats, all the good work was undone and the molds would still be present in the butter. In every case where the raw cream was cultured the bacterial count was very high and molds were present, so thorough cleaning of the whole system and care in operation was imperative.

At many different times the plates were shown to the employees and a lively interest was displayed. During the slack time there was a marked improvement in the cleanliness of the final product.

It was found that by giving the whole system a thorough steaming out before beginning the day’s run that the counts were kept down and the cream didn’t lose any of its heat while passing through its various stages; in order that the operator might know how the temperature of the holding vat was being maintained a Tycoos recording thermometer was installed. Instruction was then given in the preparation of the “mother” starter. It was found that some of the sour cream from a previous day’s run was used to make up another batch of starter and after several lots were made up in this way, a large number of objectionable bacteria would be present along with the original lactic acid culture. No definite temperature was maintained, the jars being kept between 60° and 70° in summer and between 70° and 72° in win-



ter and when the contents of the jar had coagulated the material was ready for use. This usually took seven to eight hours.

As no incubator facilities were present, it was rather difficult to devise a means to carry on the bacterial growth under methods used in a laboratory but by care in placing the jars near a steam pipe a higher temperature was obtained. Greater care was also advised in the way the milk was handled for the starter, attention being directed particularly to the pasteurization, as the milk is delivered in a filthy condition.

However, the method of using "starter" in the butter making process was discontinued and the problem of handling that item was thus disposed of.

Table II shows the condition of the cream after three months' intensive study and effort to get the working conditions improved, the ceilings, pipes, and overhead machinery cleaned, and the men trained in the habit of keeping the pipes and machinery steamed out daily and the covers closed on the ripener vats.

TABLE II

NO.	SAMPLE COLLECTED	DILUTED 10,000 TIMES		TEMPER- ATURE F	ACID- ITY %
		AGAR	1 C.C. MALTOSÉ		
5	Entering pasteurizer	1,160,000 many fine	spreaders	150-155	.43
6	Leaving pasteurizer	18,900	1 green center mold	154	
7	Leaving holding vat	8,100	1 spreader	147	
8	Bottom cooling coils			62-64	
9	Entering ripener vat	60,000		64	
13	Entering churn	10,000			
14	Buttermilk Butter	40,000	1 spreader		

Glancing at this table a marked improvement is noticed in comparison with Table I the bacterial count and mold content being greatly lowered.

During the winter months the amount of work was greatly lessened and the cream arrived in good condition as regards temperature, many cans being frozen almost solid. Greater care was also taken in the handling as only cream enough for one or two churns a day was being received. Table III is characteristic of the results at this time.

TABLE III

NO.	SAMPLE COLLECTED	DILUTED 10,000 TIMES		TEMPER- ATURE F	ACID- ITY %
		AGAR	1 C.C. MALTOSÉ		
5	Entering pasteurizer	970,000 innumerable fine	3 yeasts 1 mold	152	.43
6	Leaving pasteurizer	10,000	1 green center mold	151	
7	Leaving holding vat			149	
8	Bottom cooling coils			58-61	
9	Entering ripener	30,000		61	
13	Entering churn	20,000			
14	Buttermilk Butter	80,000			

These results follow those of Table II very closely and show what can be done when care is exercised.

After the work had been carried on for over a year another technic for sampling was adopted, namely, that of taking many small quantities from different points during the whole of the run and so making a representative composite sample of the cream at each stage in its treatment. Prior to this time but one sample was taken from each place during the course of the run, and in that way it could not be described as representative. Table IV describes the manner in which the work was handled under the changed method.

TABLE IV

SAMPLE	BACTERIA PER 1 C.C. DILUTIONS			AVE. PER 1 c.c.	MOLDS PER 1 C.C. DILUTIONS		
	1:1000	1:100	1:20		1:1000	1:20	Ave.
Raw cream	1,200,000			1,175,000	48,000		48,000
1st Comp. hold. vat	150	1000	960	980		60	60
Last " " "		1500	1400	1450		120	120
Cooler		2000	1600	1800		160	160
Churn Sam. No. I		3000	2400	2700		260	260
Churn Sam. No. II		2000	1700	1850		120	120
Buttermilk	30,000	32000		31000		280	280
Butter		8200	6500	7350		40	40

Samples 1, 2, 3, and 4 are representative of samples taken at intervals of 15 minutes during entire run from 8:30 A.M. until 11:30 A.M. Churn sample No. 1 was made up of six samples, three from each of two churns; churn sample No. 2 was made up of nine samples, three from each of three churns. The buttermilk was taken from the first churn of churn sample No. 1 as was also the sample of butter. These results are slightly high in that they show gradual increase after leaving the pastenrizer. The averages also show the enormous increase in bacteria in the buttermilk. This point should be kept in mind and followed through the data for each week.

Altogether six such comprehensive examinations were made over a period of six weeks, 43 samples in all being included in this study. By this method it was possible to arrive at an idea as to the amount of bacterial and mold removal. Table V shows this nicely.

TABLE V

SAMPLES	BACTERIA PER 1 C.C.		MOLDS PER 1 C.C.	
	1	2	1	2
Raw cream	767111	1094166	70800	45416
1st Comp't H. Vat	3700	1470	2010	39
Last Comp't H. Vat	2380	2362	1100	29
Cooler	1220	3298	1040	37
Ave. of Churn samples	2740	3565	830	51
Buttermilk	41100	36166	4950	113
Butter	6100	4958	855	24
Percentage of removal	99.01%	99.55%	98.76%	99.96%

No. 1=Samples collected from Nov. 17 to Dec. 31, 1920.

No. 2=Samples collected from Jan. 21 to Feb. 25, 1921.

There are several things of interest which may be pointed out in the disussion of the above data.

*First.*—A direct comparison of the average number of bacteria per one

cubic centimeter (Table V) in the samples collected from Nov. 17 to Dec. 31, 1920, with those of the later collections, reveals the interesting fact that although the raw cream gave a count of approximately 31,500 per 1 c.c. less than that obtained for the last collection, it also gave an end product having a count of over 1000 bacteria per 1 c.c. higher than that obtained for the later samples. In the same manner, a direct comparison of the number of molds per 1 c.c. of No. 1 with No. 2, at first sight seems to show that no great reduction has been accomplished. However, a closer study shows the reduction to have been much greater. On a directly proportional basis, if in No. 1 the raw cream with a count per 1 c.c. of 70,800 gave an end product with a count of 855, then No. 2 should give an end product having a count of 548. A glance at the table shows the count to be only 24. The percentage of removal, which is an index of plant operation, shows the same reduction. The question now naturally arises as to whether this is a true reduction or merely a "happen-to-case." As to the bacterial count, there is no doubt but that there has been a substantial reduction. As to the molds, the reduction is not so well grounded, because in some instances the dilutions were too high. Personally we do not think much weight should be attached to this objection, for even after making undue allowances, the reduction still remains very noticeable. It is evident therefore, that since there has been a substantial reduction, it can be explained only in two ways, namely, increased efficiency in plant operation, or smaller amount of raw product handled. If the amount of raw cream were checked for these two periods we believe one would find that the amount of raw product per day would be less for Jan. 21 to Feb. 25, 1921, than for the period represented by the other samples. But, after making due allowance for this fact, it must still be borne in mind that for the last period, the plant was in operation every other day, a factor which would more than offset the above argument. The only logical conclusion, therefore, is better plant operation, and from observations, we are convinced that this is the correct conclusion.

*Second.*—The manner of collecting the various samples is very satisfactory. The results given by the method of collection are very reliable and representative of actual conditions. The manner in which the raw cream is handled before it enters the pasteurizer also insures a thorough mixing before samples are taken. These samples which are taken at intervals of 15 minutes and from four different stations, give a good history of what happens to the raw cream before it is placed in the churns. Composite samples from the churns, buttermilk and butter make these samples representative of these stages.

*Third.*—The percentage of removal has been increased over that obtained for the period from Nov. 17 to Dec. 31. In case of removal of bacteria this increase is .54 per cent; in case of removal of molds, it is 1.2 per cent. Although these are apparently small, in reality they are large because after the removal has reached 99.00 per cent, 0.1 per cent means quite a noticeable increase.

*Fourth.*—The results for this period show what has been thought for

some time to be a fact, namely, a gradual increase in count after leaving the pasteurizer.

*Fifth.*—The results for each inspection, as well as the final average, conform to previous findings in that there is a tremendous increase in number, both in molds and bacteria, in the buttermilk. Of the samples collected and reported, there has been found no exception to the above statement. If this increase confined itself to the buttermilk alone, it would not be necessary to consider it, but as a matter of fact the increase is also noticeable in the butter. Reference to Table V which is representative of fifteen different inspections, shows that the count of bacteria is higher in butter than it is in the cream as it enters the churn; with the number of molds the difference is not so marked. Even so, should we add the count of the buttermilk with that of the butter, both of which are the end products of the cream, we find that the number of bacteria has been increased 13 times that found in the cream as it enters the churn, while the average increase in the molds is about five times the original number.

Nothing has been said concerning the finished product, the butter. When these studies were begun samples of butter were taken in sterile bottles and stored in the ice box for from six to eight weeks which was considered, according to office records, to be the interval which elapsed between the time the butter left the creamery until it was consumed. The object was to see whether objectionable odors, or flavors would develop and whether molds would appear. These samples were collected; possibly weekly, for one year and, with but one or two exceptions, stood the test very well. However with the improved technic adopted latterly, plates were made of the butter also.

At all times it has been noted that the buttermilk has had a high bacterial and mold content. The theory put forward by one of us and one which we feel sure will be accepted by all, is that in the process of churning all the clumps of bacteria and mold spores are broken up and so are given a greater chance to develop and so raise the count.

#### CONCLUSION

After two years' study, while the work is still incomplete and this can be called but a preliminary report, it is quite evident that by careful, efficient operation of the pasteurizer and cooling systems it is possible to produce commercially a good quality of butter of low bacterial content and quite free from molds at the time it is churned.

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# THE SYNTHESIS OF ARSPHENAMINE AND A STUDY OF SOME OF ITS INTERMEDIATE DERIVATIVES

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(Continued from page 165)

## TOXICITY STUDIES

IT HAS been observed that toxic symptoms frequently make their appearance after the administration of arspenamine. Whether this is due to an idiosyncrasy on the part of the patient, lack of the observance of proper precautions in the preparation and injection of the solution, to impurities present in the manufactured product, or to other conditions, has not been sufficiently determined to date.

The following toxicity studies were undertaken to determine to what extent, if any, undue toxicity may be attributed to the presence of certain known compounds or variations in the commercial product, and to possible contamination with certain intermediates and by-products formed in the process of manufacture.

*Arsenoxide (3-amino-4-hydroxyphenyl-arsenious oxide).*—All commercial samples of arspenamine contain a small amount (0.4 to 5.0 per cent) of material which is not precipitated from methyl alcohol by calcium carbonate. This material has been commonly referred to in the literature as "arsenoxide." A statement also occurs in the literature to the effect that this compound is twenty times as toxic as arspenamine. The latter appears to have originated through reference to an article published by Ehrlich and Bertheim in the *Berichte der deutschen chemischen Gesellschaft*. In the monograph by Ehrlich and Hata on the chemotherapy of the spirilloses, in which the data upon which this statement is apparently based are presented in detail, the information conveyed is that the tolerated dose for chickens infected with spirilloses is twenty times as great as the curative dose. For the information of the reader both articles are quoted here.

"The hydrochloride of diamino dihydroxy-arseno-benzene shares with other arsenic compounds the peculiarity that it is easily oxidizable. When it is exposed to the air, it will quickly form aminohydroxyphenylarsenoxide; however, the formation of an arsenoxide compound takes place immediately, if the experiment is carried on in the usual salt bottle. This fact is therefore of the greatest significance in the practical application of the substance as a curative agent, because the aminohydroxyphenylarsenoxide is about 20 times as toxic as the pure hydrochloride of an arseno compound. It is possible through the use of the different degrees of solubility of arseno and of the arsenoxide base to determine approximately the arsenoxide content of the preparation. (Ehrlich and Bertheim, *Berl. deutsch. Chem. Gesellsch.*, 1912, xlv, p. 764.)

"Dosis tolerata pro kilogram of chicken: 0.03 g. Amidophenol arsenoxide shows also the very good effect of this substance upon the diseased animal even much more so than upon the healthy animal. (Table xxxii.)

"If we now look over the above mentioned curative experiments with chickens infected with spirillosis, the relation between the curative dose and the tolerated dose of the single substances results as follows:

Atoxyl .....	0.03	- C -	1
	<u>0.06</u>	T	<u>2</u>
Arsacetin .....	0.03	- C -	1
	<u>0.10</u>	T	<u>3.3</u>
Arsenophenyl glycin .....	0.12	- C -	1
	<u>0.40</u>	T	<u>3.3</u>
Arsanylechloride Quicksilver .....	0.04	-	1
	<u>0.100</u>		<u>2.5</u>
Dioxydiamidoarsenobenzene .....	0.0035	- C -	1
	<u>0.200</u>	T	<u>58</u>
Amidophenolarsen oxide .....	0.0015	- C -	1
	<u>0.030</u>	T	<u>20</u>

"From the same comparison it appears that the first four remedies acted more unfavorably than the last two; of these again the dioxydiamido arsenobenzol is better than the amidophenolarsen-oxide, a fact that I was able to confirm with my experiments of relapsing fever." (Ehrlich and Hata, *Die experimentelle Chemotherapie der Spirillosen*, 1910, p. 57.)

In order to clear up the question of the toxicity of this compound, a quantity of the material was prepared and tested. Arsenic and nitrogen determinations showed that the material consisted of approximately 91 per cent of arsenoxide hydrochloride and corresponded to the formula



The product which was produced for use in this experiment contained alcohol of crystallization and the calculation for the hydrochloride is obtained as follows:



Table I shows the results of the toxicity tests made by Dr. G. B. Roth. The tests were made on rats, the material being injected in alkaline solution.

TABLE I  
TOXICITY OF ARSENOXIDE

DOSE IN MG. PER KILO	NUMBER OF ANIMALS USED	LIVED	DIED
10	5	5	0
15	5	3	2
20	5	2	3
30	5	1	4
40	5	0	5

These results show that the tolerated dose for rats lies between 20 and 30 mg. per kilo. Similar tests conducted at this laboratory with arsphenamine have demonstrated the tolerated dose to be in the neighborhood of 100 to 120 mg. per kilo. These figures correspond very closely with those obtained by Ehrlich and Hata for chickens, rats and rabbits quoted above. It may, therefore, be stated that arsenoxide is only about 6 to 7 times as toxic as arsphenamine. Furthermore, it can be stated with a fair degree of certainty that the abnormal toxicity frequently observed in the case of arsphen-

TABLE II

RELATION OF ARSENOXIDE CONTENT OF ARSPHENAMINE TO TOXICITY VALUES

PRODUCT	CONTROL NUMBER	PER CENT OF AS	PER CENT OF ARSENOXIDE
A	1	31.59	1.0
	2	31.22	0.9
	3	31.10	0.8
	4	31.27	0.6
	5	31.31	0.9
	6	31.50	0.5
	7	31.22	0.7
	8	31.31	1.7
	9	. . .	0.7
	10	31.12	1.0
	11	31.31	0.6
	12	31.50	0.5
	13	31.25	0.5
	14	30.11	0.7
	15	31.23	0.6
	16	30.75	0.5
	17	30.70	0.8
	18	30.75	0.8
	19	30.85	0.7
	20	31.12	0.8
	21	31.46	0.7
	22	. . .	1.1
	23	31.29	1.7
	24	30.60	0.5
	25	31.15	0.9
	26	30.78	0.6
	27	30.19	0.8
	28	31.25	1.0
	29	30.94	0.7
	30	31.12	1.0
	31	31.05	0.7
	32	31.09	0.7
	33	30.75	0.6
	34	31.05	1.2
	35	31.50	0.4
	36	31.44	0.5
	37	31.12	0.7
	38	31.15	0.8
	39	30.96	0.9
	40	30.63	1.8
	41	30.91	2.3
	42	31.58	1.9
	43	32.15	2.9
	44	31.97	1.9
	45	31.65	1.9
	46	30.70	1.4
	47	. . .	1.3
	48	30.92	1.5
	49	31.55	1.5
	50	30.93	1.3
	51	31.06	1.9
	52	31.48	. .
	53	30.15	. .
	54	30.30	0.8
	55	31.00	0.7
	56	31.12	0.7
	57	30.93	0.8
	58	31.20	1.6
	59	30.92	2.3
	60	31.29	2.6

TABLE II—(CONTINUED)

PRODUCT	CONTROL NUMBER	PER CENT OF AS	PER CENT OF ARSENOXIDE
Product B	1	30.54	0.7
	2	30.73	0.8
	3	30.95	0.6
	4	30.57	1.0
	5	30.49	0.8
	6	30.26	0.7
	7	. . .	0.7
	8	29.89	1.3
	9	30.88	1.1
	10	31.01	1.8
	11	30.88	1.6
	12	30.88—	1.6
	13	30.46	1.6
	14	30.65	1.6
	15	30.65	1.8
	16	30.65	1.1
	17	31.31	1.5
	18	31.01	1.8
	19	29.71	1.1
	20	30.75	1.0
	21	31.01	0.9
	22	29.89	1.4
Product C	1	30.95	1.8
	2	29.88	1.7
	3		1.9
	4	30.07	4.1
	5	30.30	1.7
	6		1.6
	7	30.53	1.3
	8	30.40	1.8
	9		1.8
	10	30.23	1.6
	11	30.49	2.3
	12	30.40	1.3
	13	30.06	2.0
	14		0.9
	15	29.75	1.5
	16		1.4
	17		1.4
	18		2.0
	19		1.1
	20		1.6
	21		2.3
	22	30.57	1.1
	23		1.4
	24	29.71	1.1
	25		1.4
	26	29.51	1.1
	27	30.75	0.8
	28	30.95	0.7
	29	30.88	1.1
	30	30.18	0.7
	31	30.75	0.7
	32	31.10	1.1
	33	30.88	1.2
	34		0.9
	35		0.7
	36		1.1
	37	31.47	1.1
	38	30.77	0.7
	39	30.94	0.9



amine is not due in any marked degree to the presence of this compound, inasmuch as numerous tests carried out in this laboratory indicate that there is no apparent relationship between the "so-called" arsenoxide content and degree of toxicity. Preparations with a relatively high arsenoxide content were found to give low toxicity values and *vice versa*. Table II contains data of this nature.

*Methyl Alcohol*.—Attention has been directed to the presence of methyl alcohol as a possible cause for abnormal toxicity. It should not be understood that the methylated salvarsans are not being referred to in this connection, but a product in which the methyl alcohol is mechanically held or is present as "so-called" alcohol of crystallization. A preparation of this nature ( $\text{HCl}$ ,  $\text{NH}_2\text{C}_6\text{H}_3(\text{OH})\text{As}$ ) $_2 \cdot \text{CH}_3\text{OH}$ ) obtained by reduction with sodium amalgam is described by Ehrlich and Bertheim (1912).

During the course of the examination of commercial samples of arspenamine in this laboratory, a number of tests were made with Schiff's reagent to detect the presence of free methyl alcohol or alcohol loosely combined. The results obtained, however, were negative in all cases. Later, 400 g. of the arspenamine were subjected to distillation and then refractionated with the result that less than 1 c.c. of methyl alcohol was obtained in the distillate. Even though this amount does not represent the total quantity present, a portion probably being lost through oxidation, it is highly improbable that there is a sufficient amount of methyl alcohol present to account for any undue

TABLE III  
HYDROGEN-ION CONCENTRATIONS OF SOLUTIONS OF THE ACID AND DISODIUM SALTS

MANUFACTURER	CONTROL NO.	P-H ACID SALT	P-H DI-NA SALT	C.C. N/1 $\text{NaOH}$ ADDED TO FORM THE DI-SALT
Product A	1000	4.8	9.4	1.44
	1239	5.1	9.4	1.47
	1208	5.0	9.4	1.70
	1303	5.2	9.4	1.72
	1294	5.0	9.4	1.36
	1288	5.2	9.4	1.52
	1292	4.6	9.5	1.36
	1300	4.4	9.3	1.50
	1298	5.0	9.2	1.45
Product B	H 28	5.0	9.4	1.72
	H 30	5.0	9.2	1.76
	H 31	4.9	9.3	1.80
	H 34	4.9	9.4	1.74
	H 37	5.0	9.3	1.72
	H 40	5.0	9.4	1.80
	H 51	4.9	9.2	1.70
Product C	A 22	4.6	9.4	1.50
	A 24	4.8	9.4	1.47
	A 25	4.8	9.6	1.47
	A 34	4.5	9.6	1.50
	A 37	4.7	9.4	1.62
Product D				
	3	4.8	9.2	1.47
	5	5.0	9.4	1.57

toxic action. In fact, when salvarsan first began to be used, it was recommended in the directions for preparing solutions for injection, that a small amount of methyl alcohol be poured over the material before the addition of the water in order to facilitate solution.

*Variations in Degree of Acidity.*—Hydrogen-ion determination made in connection with the testing of commercial samples of arsphenamine show that the hydrochloric acid content of the different preparations on the market varies to a considerable extent. This variation is thought to be the cause for the alkalinity troubles which are reported from time to time, and may be a factor indirectly influencing toxicity. It is evident that, if the hydrochloric acid content varies and the same amount of alkali is added in all cases, the hydrogen-ion concentration of the finished solutions will vary. As this condition may be an important factor influencing the formation of precipitates in the blood, its importance in this connection is readily understood. Data of this nature for solutions of the acid salt and disodium salt, using methyl red in the acid range and cresol phthalein and thymol blue in the alkaline

TABLE IV  
TOXICITY OF INTERMEDIATES AND BY-PRODUCTS

COMPOUND	PROPERTIES GENERAL	RAT	DOSE IN MGS.	DIED IN	LIVING AT END OF	REMARKS
3-amino-4-hydroxy-phenyl-arsinic acid	Light yellow powder, insol. in H <sub>2</sub> O, sol. in dil. NaOH. Sol. with a light brown color.	1904	400	46 hrs.		All in good condition
		1903	300		19 days	
		1905	200		" "	
		1906	100		14 "	
oxalyl-4-amino-phenyl-arsinic acid	Purplish-gray powder, insol. in H <sub>2</sub> O, readily sol. in weak NaOH, forming a purplish colored sol.	1962	400		13 days	
		1963	300		" "	
		1964	350		" "	
		1965	250		" "	
		1966	260		" "	
3-nitro-4-amino-phenyl-arsinic acid	Deep yellow powder insol. in H <sub>2</sub> O, sol. in dil. alkali, darkens as alkali is added forming deep mahogany-col. sol.	116	150	20 hrs.		
		115	140	3 days		
		113	140	2 days		
		114	130	4 days		
		112	110		9 days	
		92	100	4 days		
		94	90		9 days	
		93	80		9 days	
		95	70		9 days	
3:5-dinitro-4-hydroxy-phenyl-arsinic acid	Fine light yellow powder, partially sol. in H <sub>2</sub> O, forming a yellow colored sol. On making alkaline, the color changes to orange.	2065	50		14 days	
		1982	250		11 "	
		1983	300	5 hrs.		
		1984	200	9 hrs.		
		1985	150		11 "	
		1986	100		11 "	
		1972	400	6 hrs.		
3:5-dinitro-4-amino-phenyl-arsinic acid	Dark brownish yellow powder, insol. in H <sub>2</sub> O, Sol. in dil. NaOH, forming a dark brown sol.	1973	350	21 hrs.		
		1974	300	3 days		
		1975	250	22 hrs.		
		1976	200	3 days		

range, are given in Table III. The values given represent the hydrogen-ion concentrations of 2 per cent solutions of the acid and disodium salts, respectively, this being the concentration usually used in injecting rats for toxicity tests. These tests are comparable but more recent work shows that these are not absolute values.

*Intermediates and By-products.*—Several investigators have called attention to the possibility of contamination of arspenamine with certain intermediates and their derivatives, and have assumed that such contamination may be the cause of the abnormal toxicity sometimes apparently manifested by the commercial product. In order to test the correctness of this assumption, a considerable number of intermediates and by-products were isolated or prepared, and such toxicity tests carried out as conditions permitted at the time. The results of these tests show that it is improbable that the relatively small quantities of these substances, which may possibly be contained in the finished product, are the cause of abnormal toxicity.

The solutions of intermediates for injection were prepared in the same manner as in the case of arspenamine, i.e., 1.5 N/1 NaOH were added for every 200 mg. of the compound dissolved. Later investigations showed that 0.84 c.c. N/1 or 4 per cent NaOH was the proper amount of alkali to use for intravenous administration.

#### SUMMARY

The descriptions of the processes given above show that there are three well defined methods by which "nitro oxy," the mother substance of arspenamine, may be prepared. Attention has also been directed to the fact that the former, "nitro oxy," exhibits a peculiar type of dimorphism, which necessitates the exercise of extreme care in its preparation if the type best suited for reduction is to be obtained. Another important point which has been brought to notice is the importance of eliminating by-products from the intermediate substances, notwithstanding the statements to the contrary made by some inexperienced investigators, who contend that these products will be removed at some later stage in the process.

The foregoing descriptions of processes of manufacture have shown further that there are two well-defined procedures for reducing "nitro oxy" to arspenamine base, namely, the progressive and direct methods. It may also be stated that it is the latter method, or some modification thereof, which is usually followed in the preparation of arspenamine on a commercial scale. The modification most commonly made consists in the addition of magnesium chloride to the "hydrosulphite" mixture for the purpose of guarding against overreduction and sulphur compounds.

A method for converting the free base into the dihydrochloride of arspenamine has also been described. It should be stated here, however, that the process is not so simple as it appears at first glance, and that the exact technique followed in commercial practice is more or less known only to the manufacturers. It may be said in addition that the care exercised at this point is an important factor influencing the purity of the final product. "Hydrosul-

phite" contains metallic zinc and other inorganic impurities, which may be filtered off simultaneously with the free base and subsequently dissolved in the methyl alcohol-hydrochloric acid mixture if the proper precautions are not observed.

With respect to the toxicity of the preparations on the market at the present time, it may be said that the average American product is somewhat less toxic than that which was formerly produced by German manufacturers. Occasionally, however, even the American product exhibits an abnormal toxicity as shown by the routine tests on animals. This is probably due to the presence of physical conditions which have not been definitely determined to date. The results of the toxicity studies described above, however, are evidence to the effect that it is not due in any appreciable degree to the presence of arsenoxide, methyl alcohol, or the following intermediates and by-products: 3-amino-4-hydroxyphenylarsinic acid, oxalyl-4-aminophenylarsinic acid, 3-nitro-4-aminophenylarsinic acid, 3:5-dinitro-4-hydroxy-phenylarsinic acid, 3:5-dinitro-4- amino-phenylarsinic acid. Arsenoxide, although 6 to 7 times as toxic as arsphenamine, has been found in such small quantities (0.4 to 3.0 per cent) that it can hardly be considered an important factor in this connection. Furthermore, the toxicity tests carried out indicate that there is no noticeable relationship between the degree of toxicity of arsphenamine and its "arsenoxide" content. Determinations made of the methyl alcohol content show that it is also present in such small quantities that it is a negligible factor. With one possible exception, 3-nitro-4-aminophenylarsinic acid, the intermediates mentioned are less toxic than arsphenamine.

The variations observed in the hydrogen-ion concentrations of solutions of commercial samples of arsphenamine prepared by different manufacturers, using a fixed amount of alkali, indicate that the hydrochloric acid content may be a factor influencing toxicity. Animal experimentation in this field, however, will be necessary before accurate conclusions can be drawn.

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# AN EXPERIMENTAL INVESTIGATION OF THE PHARMACOLOGIC ACTIVITY OF DRUG STORE SAMPLES OF INFUSION OF DIGITALIS, U. S. P. IX\*

BY A. RICHARD BLISS, JR., PHM.D., M.D., ATLANTA, GA.

BECAUSE of the frequent complaints of clinicians concerning the unreliability of infusion of digitalis, U. S. P., and the meager available data concerning the relative activity of this preparation, particularly the preparation as it is found in the average retail pharmacy, the writer undertook the investigations the results of which are herewith presented.

The United States Pharmacopeia gives biologic assays for digitalis,<sup>1</sup> fluid-extract of digitalis,<sup>2</sup> and tincture of digitalis,<sup>3</sup> but gives no method for standardizing infusion of digitalis.<sup>4</sup> One reason for the omission of a biologic assay in the case of the infusion is indicated in the pharmacopeial instructions "*Infusion of digitalis must be freshly prepared from the leaves,*"<sup>5</sup> it being doubtless taken for granted that an infusion *freshly* made according to the pharmacopeial method from standardized leaves will represent the activity of the amount of standard leaves employed. The results of this study will be of interest to those who formulated the foregoing conclusion.

Some years ago many schools taught that the infusion of digitalis was a more active diuretic than the tincture and the fluidextract, explaining this supposed difference on the ground that the several active constituents of the crude drug are extracted in different relative proportions by the menstrua employed in the infusion<sup>6</sup> (water), the fluidextract<sup>7</sup> (5 of alcohol to 1 of water—by volume), and the tincture<sup>8</sup> (3 of alcohol to 1 of water—by volume). Although clinical experiences, animal experimentation, and the physical properties of the active constituents of digitalis long ago showed the errors of such belief, there are many practitioners who are seemingly firm believers in this "theory." Digitoxin, digitalin, and digitalein, the three glucosides that supposedly represent the activity of digitalis, are all soluble in alcohol. Digitoxin, which according to Bastedo<sup>9</sup> and others most nearly represents the digitalis actions, is practically insoluble in water; digitalin is slightly soluble in water; and digitalein is soluble in water. Cushny<sup>10</sup> states that infusion of digitalis contains only traces of digitoxin. Sollmann<sup>11</sup> states that a 1:10 infusion (not U. S. P.) contains  $\frac{2}{3}$  of the digitoxin of the leaves. A fourth principle, a saponin body called digitonin, is soluble in water, and is said by Bastedo<sup>12</sup> and others to possess the property of holding the otherwise water-insoluble (or slightly soluble) glucosides of digitalis in aqueous solution. It is also stated that digitonin, by the intravenous method, is a physiologic antagonist to digitoxin, but that it is unabsorbable by the alimentary tract. Kiliani<sup>13</sup> claims

\*From the Laboratories of Pharmacology of the School of Medicine of Emory University.  
Received for publication, June 25, 1921.

that the crude drug contains but traces of digitonin. Bastedo<sup>14</sup> says he has frequently seen the infusion given in one-half ounce doses, the equivalent of 36 minims of the tincture. The writer has seen one ounce doses of the infusion (U.S.P. VIII) administered without subsequent toxic symptoms.

Hatcher and Eggleston<sup>15</sup> showed that an infusion made by pouring 1000 c.c. of boiling water onto 10 grams of digitalis (No. 60 powder), allowing it to stand in a boiling water-bath for one hour with frequent stirring, and filtering while hot, retains its activity with little loss for several weeks. Pittinger and Mulford<sup>16</sup> showed an average loss of 47.8 per cent in five samples, 8 months old, of a 50 per cent alcohol, not defatted tincture; 22.8 per cent loss in five samples, 8 months old, of a 50 per cent alcohol, fat-free tincture; and 40.7 per cent loss in five samples, 8 months old, of an 80 per cent alcohol, fat-free tincture. They present no data concerning the infusion. Hale<sup>17</sup> found official fluidextracts to have lost only an average of 6.6 per cent in two years. Roth<sup>18</sup> found an average loss of activity in seven samples of fat-free tincture of 14 per cent in six months; two of his samples showing no loss, while two others showed a very high loss. Houghton and Hamilton<sup>19</sup> showed an average yearly loss of 8 per cent in eleven samples of extract five years old; 4 per cent average yearly loss in eight samples of fluidextract, (U.S.P. VII,) six years old; 10 per cent average yearly loss in eleven samples of fluidextract, (U.S.P. VIII,) three and one-half years old; and 9 per cent average yearly loss in eight samples of tincture three years old. They concluded that a maximum average loss of 10 per cent per year can be expected in the tincture and the fluidextract. Goodall<sup>20</sup> states that probably tincture of digitalis retains its full activity for one year. Hamilton and Rowe,<sup>21</sup> following a series of experiments with the tincture which showed a loss of from 0 per cent (with fat-free preparations) to 43 per cent, the ages running from 5 months to 8 months, concluded that the degree of deterioration varies with different lots, that a fat-free tincture made with 70 per cent alcohol is less subject to deterioration, and that the deterioration of the tincture is not as uniformly rapid as isolated experiments would indicate. Bastedo<sup>22</sup> states that under the influence of heat or acids, or when kept for some time in aqueous solution, as in the case of the infusion, the glucosides of digitalis tend to decompose and may form toxiresins which have a central convulsant action. None of the foregoing investigators, with the exception of Hatcher and Eggleston, present data concerning the stability and the relative activity of the infusion.

#### THE METHOD

The method of biologic standardization used in this investigation is that known as the Hatcher and Brody Cat Method.<sup>23</sup>

#### THE SAMPLES

The samples investigated were obtained from various retail pharmacies.

#### THE RESULTS

The results of the investigation are presented in tabulated form. The values in the last column of the table represent the activities of the samples



expressed as percentage of the theoretical activity as calculated from the amounts of standardized drug supposedly used.

TABLE I

TABLE SHOWING THE RELATIVE ACTIVITY OF VARIOUS SAMPLES OF INFUSION OF DIGITALIS OBTAINED FROM RETAIL PHARMACIES

The values in the "Average Activity" column express the percentages of the theoretical activity as calculated from the amount of crude drug employed or supposed to have been employed. Fractions are omitted.

SAMPLE	METHOD OF PREPARATION	NUMBER OF ESTIMATIONS	AVERAGE ACTIVITY (PERCENTAGE OF THEORETICAL ACTIVITY)
1	U. S. P. IX	4	31%
2	U. S. P. IX	4	42%
3	Dilution of Fluidextract	4	60%
4	Dilution of Fluidextract	3	65%
5	U. S. P. IX	4	33%
6	U. S. P. IX	4	51%
7	U. S. P. IX	3	29%
8	Dilution of Fluidextract	4	63%
9	Dilution of Fluidextract	4	60%
10	U. S. P. IX	4	37%
11	U. S. P. IX	4	40%
12	Dilution of Fluidextract	3	65%
13	U. S. P. IX	3	42%
14	U. S. P. IX	4	41%
15	U. S. P. IX	4	35%

## SUMMARY

Fifteen samples of infusion of digitalis, selected at random from retail pharmacies, showed an average activity of but 46.26 per cent of the theoretical activity calculated from the amount of standardized drug supposedly used in the manufacture of the infusion.

Five of the fifteen samples, prepared by a method that is frowned upon by the medical and pharmaceutical professions (simple dilution of the fluidextract), showed an average activity of 62.6 per cent; or 16.34 per cent stronger than the average for the total fifteen samples, and 24.5 per cent stronger than the ten samples supposedly prepared by the U.S.P. IX method.

The ten samples manufactured according to the method of the U.S.P. IX showed an average activity of but 38.1 per cent.

The results obtained are interpreted as indicating:

(a) A rather general variability of the U.S.P. infusion, all samples examined falling well below the theoretical activity.

(b) A decidedly more active "infusion" when made by dilution of the fluidextract than when prepared by the method of the U.S.P. IX.

(c) The need for an improved method for the preparation of infusion of digitalis. The present official method undoubtedly calls for an insufficient amount of solvent actually employed for extraction, too short a period of infusion, and an insufficiently fine powder.

(d) The need for standardizing the infusion. Many will doubtless look upon this as theoretically desirable, but at the same time impracticable.

(e) The fact that the present infusion of digitalis might be dropped from the U. S. P. without handicapping in any way modern therapy. There is serious doubt whether a standard infusion possesses any advantages over a standard tincture. Laboratory investigations and clinical experiences have shown that the tincture is decidedly more uniform, reliable, and stable than the infusion.

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## CHRONIC NEPHRITIS WITH AN UNUSUAL DEGREE OF NITROGEN RETENTION\*

BY EDWARD WEISS, M.D., AND VAUGHN C. GARNER, M.D., PHILADELPHIA, PA.

THE patient, a white male, age 37, a policeman for the past fifteen years, was admitted to the Jefferson Hospital on the service of Prof. H. A. Hare, April 12, 1921, complaining of shortness of breath.

His family history was negative. He had typhoid fever at thirteen, gonorrhea at twenty-two, and influenza at thirty-three (in 1917). From these infections he apparently made a good recovery.

Seven weeks prior to admission he was suddenly seized with dyspnea during the night. This nocturnal dyspnea continued, but up to the time of his admission to the hospital he had been free from discomfort during the day. The only other point of importance in his history was a nocturia of two or three years' standing—he stated that he had to get up one or two times every night to urinate.

Physical examination showed a fairly well nourished, adult, white male with marked pallor. The area of cardiac dullness was somewhat increased to the left; his blood pressure on admission was 220 systolic and 180 diastolic; there was no edema of the extremities. Urinalysis showed a cloud of albumin and a few hyaline and granular casts. Hemoglobin was 56 per cent; Wassermann (blood and spinal fluid) was negative. Eye grounds showed a moderate albuminuric retinitis. The phenolsulphonephthalein test gave only traces of dye elimination in two hours. The two hour test showed a fixation of specific gravity at about 1.011 and a total volume in 24 hours of 1080 c.c.

His general condition grew steadily worse; his nocturnal dyspnea increased in severity. April 19 (seven days after admission) he became drowsy and developed a persistent diarrhea which continued for three days before yielding to treatment. He vomited occasionally and on April 26 a pericardial friction rub was heard. May 1 there was a decided change for the worse and death occurred on May 4.

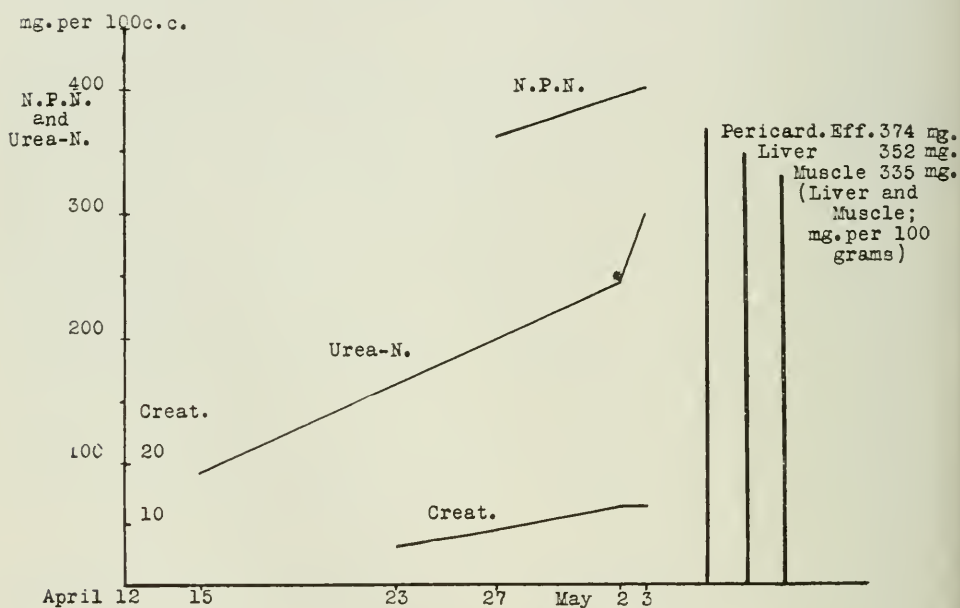
Of greatest help from a prognostic standpoint were the estimations for nitrogen retention in the blood. These are shown in the accompanying chart. With an initial blood urea-nitrogen figure of 93 mg. this gradually rose and 12 hours before death reached the astounding figure of 304 mg. The creatinine (13 mg.) and nonprotein nitrogen (401 mg.) paralleled this remarkable rise. The asterisk on the chart denotes an estimation of urea-nitrogen (252 mg.) in the spinal fluid. This was found to coincide almost exactly with the blood estimation (246 mg.) done at the same time.

\*From the Department of Pathology and Medicine, Jefferson Medical College, Philadelphia, Pa.  
Read before the Section on General Medicine of the College of Physicians of Philadelphia, May 30, 1921.

Necropsy was performed the day of death and showed a marked chronic interstitial nephritis, arterial disease and left-sided cardiac hypertrophy with an ulcerative colitis, probably of the so-called uremic type, and serofibrinous pericarditis.

The kidneys weighed but 80 grams each and did not present quite the usual picture of marked chronic nephritis, in that they were not so coarsely granular; advanced scarring was not a feature and there was no increase of pelvic fat; in other words, the kidneys seemed naturally small rather than atrophic. It was felt that, with no etiologic factor discoverable in history or physical examination, such an advanced degree of nephritis in a young man

**An Unusual Degree of Nitrogen Retention  
in the Body Tissues and Fluids in a  
Case of Chronic Nephritis.**



\* Urea-N. of the spinal fluid.

Fig. 1

might point to a congenital basis, in spite of the fact that no definite proof could be offered for this suggestion by the gross appearance of the kidneys.

After death the pericardial effusion, liver and muscle were analyzed for their urea content (denoted by the solid columns on the chart) and it is obvious from the heights of these columns that they approximate closely the blood urea figure (304 mg.) of 12 hours before death—being naturally a little higher because of the time elapsing. This indicates the approximately equal distribution of urea in all the body tissues and fluids—for here we have an estimation of the blood and spinal fluid and later of an inflammatory exudate (pericardial effusion) liver and muscle.



## COMMENT

The results of the estimations of nitrogen retention in the various fluids and tissues of this patient agree with the few recorded instances in literature. Marshall and Davis<sup>1</sup> in a study of the distribution of urea analyzed (1) tissue before and after injection of large amounts of urea; (2) tissues of animals whose kidney function had been interfered with—with or without urea injection; and (3) human tissues from necropsies on nephritics. They found the urea content of all organs and tissues approximately uniform and about equal to that of the blood, both in normal conditions and when an abnormally large amount of urea was present. Their experiments show that urea is nontoxic in any moderate or fairly large amount. "Only when introduced in enormous doses—in the neighborhood of 1000 mg. per 100 grams of body weight—does it produce a fatal effect." Therefore, they feel that it is improbable that urea is the only toxic agent in uremia but believe it may take some share in producing the symptoms of the condition. It should be remembered—they add—that urea is equally increased in the central nervous system and at all other points. Our analyses, in the one case at least, bear out this assertion.

Foster<sup>2</sup> in an estimation of the extract-nitrogen of the tissues in chronic nephritis found it to be increased to 50 per cent or more above normal—which he states is always less than 1 gram per 100 grams of dry substance.

Hewlett, Gilbert and Wickett<sup>3</sup> in a noteworthy experiment to determine the toxic effect of urea on normal individuals found that by administering large doses (100-125 grams), symptoms comparable to those encountered in the asthenic types of uremia could be produced. This indicates to them that the high concentration of urea in the body tissues and fluids in nephritis is an explanation in part of the bodily and mental asthenia, but by no means excludes the possibility that other substances may play a rôle in producing the symptoms of this type of uremia, and indeed, the fact that animals die only when extraordinary doses of urea are given suggests that the fatal outcome of asthenic uremia in man may be due to other substances than urea.

Many other observers subscribe to this belief and the remarkable figures for nitrogen retention in states of anuria, without symptoms of uremia, are well known. Only recently Scholl and Foulds<sup>4</sup> recorded a case of prolonged anuria with a urea retention of 528 mg. per 100 c.c.

On the other hand, there is the excellent suggestion of Wells<sup>5</sup> that the reported experimentally determined toxicities with nitrogenous substances have represented only transitory conditions which are entirely dissimilar to the actual conditions in the body. These experiments correspond to the cases of bichloride poisoning, etc., with high nonprotein nitrogen in the blood, in which absence of the uremic symptom complex has been noted and remarked upon. He feels that to study the relation of uremia to retained metabolites, we need observations on their effects when maintained in the organism for long periods at the concentrations occurring in uremias.

## SUMMARY

A case is presented of severe chronic nephritis in a young man, which ran a short, fatal course. There was no definite etiologic factor and the thought was suggested that there may have been a congenital basis. The case was marked by an unusual degree of nitrogen retention in the various fluids and tissues of the body. The urea was found rather uniformly distributed and approximately equal to that of the blood (304 mg. per 100 c.c.).

It is felt that if urea is not responsible for the uremic symptoms, it at least serves in many cases as a valuable index to the poisonous substance or substances that are responsible.

We are indebted to Prof. H. A. Hare for permission to publish this case and to Dr. L. A. Hamilton for assistance with laboratory determinations.

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# MERCURIC CHLORIDE POISONING WITH RECOVERY

## A CASE REPORT WITH A NOTE ON THE UREA-CONCENTRATION TEST\*

BY ELMER H. FUNK, M.D., AND EDWARD WEISS, M.D., PHILADELPHIA, PA.

**B**ICHLORIDE poisoning with recovery, as a result of the modern intensive treatment, is no longer of unusual occurrence.<sup>1</sup> The present case is recorded, however, in order to present the functional studies, which include the urea-concentration test, and in that connection to continue a discussion outlined in a recent case report,<sup>2</sup> of the role of urea-retention in the production of asthenic uremia.

### CASE HISTORY

J. T., a white male adult, aged 33, was admitted to the Jefferson Hospital, service of Dr. McCrae, July 21, 1920. On Thursday night July 15th, he had taken four tablets of bichloride of mercury, with suicidal intent. He vomited in less than five minutes and continued to vomit throughout the night. By the next day (Friday) vomiting had ceased but his bowels moved very freely. Saturday and Sunday he was up and about but did not feel able to go to work; Monday, July 19th, he noted that his urine was dark colored and scanty and from then to the time of his admission to the hospital, Wednesday, July 21st, he stated that he had not passed any urine. He applied for admission because of a return of nausea.

*Condition on Admission.*—A fairly well nourished adult male, mentally clear, who did not appear very sick. The only point of importance in the physical examination was the condition of the mouth; the gums were red and tender and bleeding. He was immediately placed upon the Lambert-Patterson treatment and the subsequent course of the case can best be traced by reference to the chart.

The solid black line, representing the total excretion of urine in c.c. per day, shows 3 days of anuria. Two of these 3 days of anuria occurred before admission to the hospital. On the second day after admission, July 22, he excreted 50 c.c., then 100 c.c. for each of three days; followed by a sharply ascending scale until on August 12, 24 days after admission, he was excreting between 4000 and 5000 c.c. per day and maintained this level until August 27; from then a general decline until upon discharge on September 14, he was excreting a little less than 3000 c.c. a day.

The blood urea-nitrogen began at 140 mg. per 100 c.c. on the 6th day after admission, July 24. This rose to 176 mg. on July 30, and from then a gradual decline in the curve occurred to a normal blood urea-nitrogen (18 mg.) on the day of discharge.

During his days of low total excretion it was impossible to test his phenolsulphonephthalein elimination due to the fact that he secreted no urine during the period of the test. On the seventh day after admission, July 27,

\*From the Departments of Medicine and Pathology of the Jefferson Medical College.  
Received for publication, July 7, 1921.

his phthalein excretion could be considered a mere trace, and his next estimation on July 31 was no more. From then on the phthalein elimination gradually rose until on the day of discharge it was 45 per cent.

A new method of estimating renal function was used upon this patient.—the Urea-Concentration test of MacLean and DeWesselow,<sup>3</sup> two English observers. This test depends upon the decreased concentrating power of a diseased kidney and is performed in the following manner: fifteen gm. of urea dissolved in 100 c.c. of water, flavored with tincture of orange, are given by mouth after the patient voids. Urine is passed at the end of one hour and at the end of two hours, both specimens being measured and the second, MacLean and Russel,<sup>4</sup> saved for analysis. In those cases showing an excretion of from 350 to 600 c.c. or more in the two hours, any tendency to a low concentration may be put down to excessive fluid, not to kidney

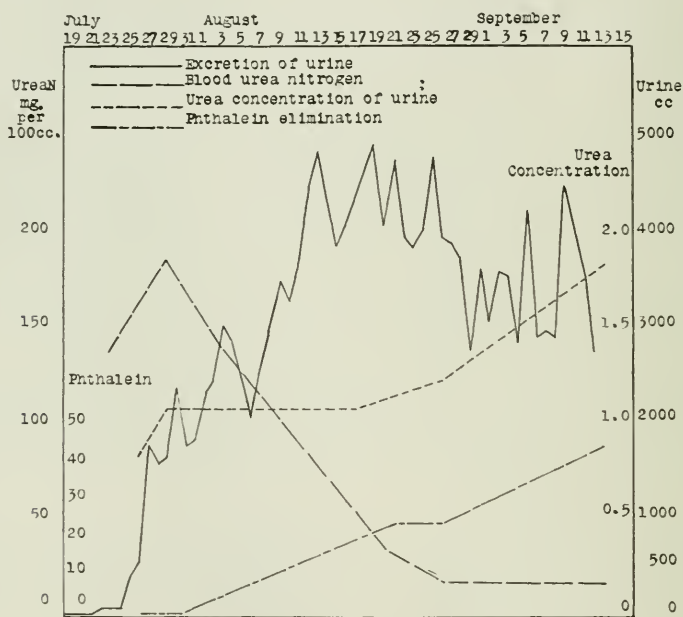


Fig. 1

disease. If the individual can concentrate urea in the second hour specimen to 2 per cent or better, his kidneys are considered efficient; if less than 2 per cent, diseased; and the lower the concentration, the greater the structural damage. It is noted in the present case that a marked parallelism exists between the urea-concentration and the phthalein elimination; the urea-concentration beginning at 0.85 per cent July 27th, and ending at 1.85 per cent September 14th; the latter figure is considered slightly less than normal.

This test has proved a valuable aid in the determination of the efficiency of the kidneys and in a preliminary report it was noted by one of us<sup>5</sup> that there is a close parallelism between the urea-concentration and the phthalein elimination in various grades of chronic nephritis. However, an occasional patient with mild or moderately advanced chronic nephritis, who is capable of normal



phthalein elimination, fails to concentrate urea. This is thought to depend upon one of several advantages which urea possesses as a test substance for kidney function, i.e., the administration of a large dose of urea throws a strain upon the kidneys and thus brings to light latent deficiencies of these organs.

In the present case, though the test gave no more information than the phthalein elimination or the blood urea estimations, it did furnish some interesting data for comment and speculation as follows:

Lewis and Rivers<sup>6</sup> in a report of a case of bichloride poisoning with complete chemical studies, state in their summary of the case that the retention of waste nitrogen is undoubtedly a factor in the early fatal issue of these cases. Though the height of the nitrogen retention can usually be relied upon as a prognostic sign of importance in these and similar states of anuria, it is doubtful if we have any real proof, any more than we do in true uremia, that the urea or other nitrogen products are the substances actually responsible for the deaths of these patients.

In the present case the 15 gm. of urea administered to the patient on July 27th, in the performance of the urea-concentration test, raised the blood urea nitrogen from 156 mg. to 171 mg. per 100 c.c. in a period of 2½ hours, at which time the maximum blood concentration following urea administration is said to occur.<sup>7</sup> This may have been largely responsible for the high figure, 176 mg., of the next estimation, July 30th, for this test raised the blood urea figure to a similar degree and the same is true of all the subsequent tests. Here then, we had a pathologic accumulation of urea, and similar products, to an unusual degree, augmented by the administration of a large dose of urea on five different occasions. In spite of this procedure, the blood urea curve steadily fell to normal; the patient never showed any untoward symptom which could be attributed to the administration of urea, and on September 15th, 57 days after admission, he was discharged apparently well.\*

Though this appears to uphold the contention of many other observers that urea is, for practical purposes, an innocuous substance; and to cast doubt upon the belief that urea, or its allied products may be responsible for certain types of uremia; still, it is well again to call attention to the statement made by Wells<sup>8</sup> that in our efforts to produce uremia experimentally we have failed to maintain urea and its allied substances in the animal body at the constant concentrations over a long period of time at which they occur in many cases of uremia in man.

#### SUMMARY

A case of bichloride poisoning with recovery following intensive eliminative treatment, is presented.

Functional studies include the urea-concentration test which has been found of more value in the study of chronic nephritis but in the present in-

\*The patient returned three months after his discharge from the hospital, Dec. 21, 1920, and at that time presented the following findings: heart, normal in size and function; blood pressure 104/70; eye grounds negative; urine, specific gravity 1023, very faint trace of albumin, an occasional hyaline cast in the centrifuged specimen; blood urea-nitrogen 15 mg. per 100 c.c.; phthalein elimination 60 per cent in two hours; urea-concentration 2.6 per cent.

stance, as an interesting side-light, furnished information as to the comparative innocuousness of administered urea. If the urea-concentration test is to be more generally adopted this observation is of importance.

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# LABORATORY METHODS

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## A NEW MICROCOLORIMETER\*

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MICROCOLORIMETRIC methods which permit the estimation of such blood constituents as hemoglobin, sugar, urea, etc., in the small quantities of blood obtainable from the finger tip or ear lobe have made a strong appeal to many clinical laboratory workers. Although these individuals have conceded that the pain incident to securing blood may often be greater than when larger quantities of blood are properly taken by venipuncture, they have continued to seek micro methods, despite their recognized inferior accuracy. One of the greatest sources of error in these methods has been in the type of colorimeter employed.

The Sahli-Gower hemoglobinometer is probably the most common form of microcolorimeter. Epstein<sup>1</sup> has utilized this instrument in the estimation of blood sugar, while more recently Kuttner<sup>2</sup> has employed the instrument, slightly modified, for a number of micro estimations. Kuttner's instrument differs from the original instrument chiefly in that Helmholtz prisms have been introduced to secure better comparison of the standard and unknown. In this type of instrument the unknown is brought into color harmony with the standard by dilution with water or some diluting fluid. As the writer<sup>3</sup> has previously pointed out, the principle of this method is excellent, and perfectly satisfactory results may be obtained, provided fairly large volumes of fluid are employed (10 c.c. or more) and care is taken in making the dilution. In such small instruments as the Sahli or Kuttner, however, one or two drops, more or less, result in a very considerable error.

With graduated tubes of suitable caliber it is possible to make quite accurate dilutions to such small volumes as 1.5 c.c. How may the color intensity of such small volumes be compared? One does not require a so-called microcolorimeter to make the color comparisons with the micro methods. It is quite possible to make color estimations with 2 c.c. or even 1.5 c.c. of fluid in the Hellige or Bock-Benedict colorimeter, provided that with the latter instrument, the lumen of the cup is nearly the same size as the plunger. Such a cup may also be obtained with the Elei simplified Duboseq. Obviously the various other instruments of the Duboseq pattern can likewise be adapted to this work, although most of these instruments are too expensive to find common clinical use.

Kleiner<sup>4</sup> has recently described a microcolorimeter on the wedge prin-

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eiple, which obviates the dilution of the unknown required for the matching of the standard in the Sahli type of instrument. In the Kleiner instrument the wedge is made by grinding a small bore test tube at a definite angle and fusing it to a ground glass plate. This is used for the standard. When accurately calibrated, good results may be obtained with this instrument, but the difficulty of making two wedges alike is apparent.

In the colorimeter to be described it is believed that this difficulty has been overcome. The instrument is somewhat similar in principle to the Helige, in which a hollow glass wedge is employed to hold the standard. In the present instrument\* a solid wedge of colorless optical glass is fused in the bottom of a test tube of uniform bore. Obviously if the ground portion of the wedge is 100 mm. in length and tapers from the diameter of the tube to 0

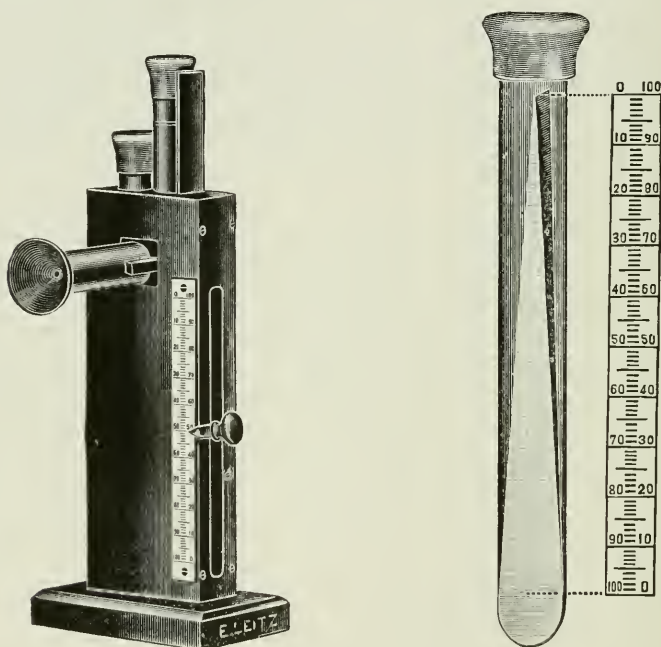


Fig. 1.—Microcolorimeter with accessory wedge to illustrate the principle of the instrument.

mm. and a 100 mm. scale is set on the instrument to correspond with the wedge, (see Fig. 1), a solution  $\frac{3}{4}$  the strength of the standard will read 75, a solution  $\frac{1}{2}$  the strength 50,  $\frac{1}{4}$ , 25, etc., thus making the calculation very simple, since the readings give the strength of the unknown in per cent of the strength of the standard.

The instrument illustrated above is about 210 mm. in height from bottom to top of tubes, the box without base having a height of 150 mm. The tubes which have been employed have a bore slightly greater than 11 mm., the wedge tube being 150 mm. in length and the tube for the unknown 75 mm. The tube

\*The author is indebted to Mr. Walter C. Freisinger, of E. Leitz, for suggesting the use of a solid glass wedge and other help in connection with the construction of the instrument. The instrument is being manufactured by E. Leitz, New York.



for the standard requires 5 c.c. of fluid to cover the top of the wedge, while readings may be made with 1.5 c.c. (and even 1 c.c.) in the tube for the unknown. This tube is graduated at 1.5, 3 and 5 c.c. The 1.0 and 1.5 c.c. dilutions can best be made in a graduated tube of about 6 mm. bore, and the solution then poured in the colorimeter tube.

The instrument is provided with Helmholtz prisms about 4 mm. in thickness, and an eye piece 60 mm. in length containing a lens which increases the size of the field nearly three times (to about  $8 \times 14$  mm.). The light is allowed to pass through a  $25 \times 50$  mm. milk glass plate at the back of the instrument, the tubes being separated, and prisms so set, that the light must pass through the center of the tubes. A collar is fitted to the wedge tube so that the wedge must set at a right angle to the light. The tube is provided with a very simple arrangement for moving it up and down so as to bring it into color harmony with the unknown. A pointer indicates the height of the tube on the scale.

The calibration of the wedge tube is very simple. Some stable standard with a good color is taken and dilutions prepared of 25, 37.5, 50, 62.5 and 75 per cent of the standard. Readings are taken and plotted. They should all fall in a straight line. If the wedge is correct for the tube, the readings will be identical with the percentage dilutions. A slight divergence, 1 or 2 points, may be overcome by an adjustment of the scale. If the divergence is more than this, calculation of results may easily be made with the aid of the plane curve which has been plotted.

Standards which are fairly permanent may be left in the wedge tube or fresh standards may be prepared at the time of the test.

The instrument has been employed for micro blood sugar estimations following the micro Benedict technic given by Kleiner<sup>4</sup> and perfectly satisfactory results have been obtained. It should work equally well for hemoglobin, urea, etc. Further discussion of the uses of the instrument will be given at another time.

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## SOME TIME-SAVING DEVICES FOR HANDLING PARAFFIN EMBEDDED MATERIAL\*

BY R. C. WHITMAN, M.D., BOULDER, COLO.

IN THE Department of Pathology of the University of Colorado, all the material for the class in general pathology is embedded in paraffin, except in the few cases where celloidin embedding is especially indicated. The sections are stained and covered for the class and the sections become the permanent property of the student. The labor incident to the preparation of so much material has stimulated us to seek short cuts and labor saving devices to the greatest possible extent. Among these, the following methods, which we have found most useful, are offered here in the hope that they may be of service to others.

### 1. PREPARATION OF PARAFFIN

We have found that the addition of about 5 per cent white beeswax, (best melted separately, and mixed with the melted paraffin), to the paraffin, as recommended by Grass, in the *Enzyklopadie der mikroskopischen Technik*, ed. 2, ii, p. 371, renders the paraffin tougher and less brittle.

### 2. MOUNTING OF SECTIONS

The ribbons of sections are laid on water heated to about 44° in an automatically regulated water-bath. To the water is added white of egg in the proportion of half the white of an egg to six liters of water. A crystal of thymol or carbolic acid, about 1-1000, will prevent the growth of bacteria in the water-bath. When the surface of the water becomes covered by remnants of sections or bubbles, it can be skimmed clean with a sheet of the paper towelling so generally used. Loss by evaporation is made up simply by adding more water. The albumin solution is replaced by fresh solution about once a month.

(The same paper towelling, by the way, we have found to be a cheap and satisfactory filter paper for filtering such things as hematoxylin solutions).

The sections are cut apart with a scalpel, slightly heated in the flame. If the scalpel is too hot, it will melt the sections. If it is not hot enough, the sections will stick to the scalpel. A little practice will enable one to judge the correct temperature very easily. The sections are picked up from the surface of the water on clean slides without the use of any special fixative, the white of egg in the water-bath taking the place of the customary albumin fixative.

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### 3. DRYING THE SECTIONS

For this we use racks made as follows: Aluminum combs, with teeth far enough apart so that slides will go between the teeth, are fastened side by side two and a quarter inches apart by means of copper tubing such as is used in garages for gas and oil lines. The lengths of copper tubing are placed between the combs, and the combs held in place by stove bolts, which pass through the combs and the copper tubing. Handles made of fairly stiff tinned iron wire are soldered to the copper tubing. The upper end of the handle is bent in a hook to hold a tag. Slides carrying the wet sections are placed in these racks, tagged, and placed in a cupboard heated by electric lamps to dry. As there is no glycerine on the slides, they will dry in an hour or two, and be ready for staining.

### 4. STAINING

The rack carrying the dry slides is placed in a dish large enough to hold the rack. Formerly, we used the so-called German butter jars, but since these can no longer be obtained, we use dishes made at a local tin shop from copper or tin. The rack of slides is carried as one slide through a series of such dishes holding the necessary xylol, alcohol, water, etc., into the stains, washed, and again passed to the xylol, all as one slide.

### 5. MOUNTING

The laboratory staff is unanimously agreed that one of the most useful labor saving devices we have worked out consists in the use of an ordinary medicine dropper in the balsam bottle, instead of the customary glass rod. The racks of slides when stained, washed, dehydrated, and cleared are lifted from the xylol and allowed to drain for a moment; the slides laid out on filter paper in groups of ten or a dozen, blotted lightly, covered with balsam by means of the quick-acting dropper, and cover glasses dropped in place. The slides are then picked up and placed in slide boxes. They are then placed in the paraffin oven overnight to dry, and are then put away until needed by the class.

The laboratory staff is agreed that by these methods, cutting sections in paraffin becomes much less time-consuming and laborious than cutting and staining in celloidin.

The most laborious part of the paraffin work remains, of course, the wiping of slides and cover glasses, which, when celloidin is used, is ordinarily done by the student; but a student assistant, working an hour or two a day, keeps us supplied with slides and cover glasses.

# *The Journal of Laboratory and Clinical Medicine*

VOL. VII.

JANUARY, 1922

No. 4

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Washington, D. C.

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## EDITORIALS

### *The Nature of Flutter and Fibrillation of the Auricle*

THE commonly accepted explanation of the phenomenon of auricular fibrillation is that the auricular tissue is as a whole broken up into lesser or greater numbers of small areas, each of which independently and spontaneously elaborates heterogenous impulses. The impulses bombarding the ventricular mechanism come at absolutely irregular intervals from scattered and diverse places in the auricles. In fibrillation the ventricles contract absolutely irregularly, without dominant rhythm, and the auricle is described as being in a condition of delirium. In flutter, on the contrary, the auricle beats quite regularly but at a very rapid rate, much more rapidly than does the ventricle.

Thomas Lewis has recently reported experimental work which clarifies greatly our knowledge both of auricular fibrillation and of auricular flutter. The interpretation of these conditions is to be found in the so-called ring experiment, first performed by Mayer in 1908. The experiment has since been repeated on various types of muscle and has been amply corroborated. The important points are as follows.



If a piece of muscle be so cut as to form a circular band, entirely continuous, but without muscular tissue at the center, electrical stimulation at one point will cause a wave of muscular contraction to spread around the band in each direction, the two waves meeting at a point farthest away from the area of stimulation. Here they cease. The spread is equally rapid in both directions. Fibres which have just contracted remaining temporarily refractory to renewed stimulation, the two waves after meeting do not pass each other and do not continue on around.

Repeated rhythmic stimulation of the muscular band, if timed slowly enough so that the fibres may recover their irritability, will result in repeated rhythmic contraction,—waves beginning at the point of stimulation and spreading in both directions around to the far segment of the circle. It is even possible to start a new wave before the preceding one has completed its circuit, provided the muscle at the point of stimulation has already regained its original irritability. If the frequency of stimulation be increased, the intervals between waves become progressively lessened, until eventually stimuli are applied to muscle which has not completely recovered its responsiveness. Under these circumstances the waves cannot travel with customary freedom in all directions. Occasionally, if one portion of the muscle is particularly unresponsive the wave will be found to travel only in one direction. It will spread in both directions until it meets the unresponsive segment, where one crest will die out, while the other continues on around, in the opposite direction.

If, precisely at this point, with but one wave traveling around the band of muscle tissue, the series of stimulations be discontinued, the wave will continue on around and around, indefinitely. When it reaches a point 180 degrees from the point of starting there will be no wave to meet it and stop its progress, and it will continue on back around the other half of the circle. It will continue on, past the place where the symmetrical wave died out if the fibres have had time to regain their responsiveness. Progressing around the entire ring it comes back to the starting point, to find that this portion of the muscle also has recovered. The one crest is now free to circulate indefinitely, for as it reaches each new segment of the ring it finds that segment again excitable. It proceeds around and around so long as the conditions remain unaltered, and continues its course as a circulating wave without ending.

“Thus it has been shown that, the conditions being rightly controlled, the last stimulus of a series may originate, not a single response of the ring, but an unlimited series of responses. Mines saw the wave continue to circulate for minutes. Mayer saw it continue for many hours; this is what is meant by circus movement; it is constituted by a wave of response, which travels continuously along a re-entrant path of muscle.”

In auricular flutter the auricles beat regularly at a rate of from 230 to 350 per minute. The ventricles usually contract at about half the auricular rate,—sometimes a smaller fraction, and are also usually regular, with an occasional jump to the full auricular rate. Lewis has produced flutter in dogs by rhythmic stimulation of the auricle with induction shocks given at a rate of 300 to 600 per minute. The auricle is driven rhythmically and rapidly for

a few seconds and the stimuli are then abruptly withdrawn. In a certain number of such experiments a rapid action of the auricle continues after stimulation has ceased. In some, a typical auricular flutter will thus be produced.

Lewis has mapped out the path taken by the excitation waves both under normal conditions and while flutter is in progress. Normally this wave starts in the sino-auricular node or "pacemaker," situated at the junction between the superior vena cava and the right auricle. From here it spreads simultaneously in all directions, and around both venæ cavæ. The impulses, passing around this circle of veins, meet at the lower point and fuse.

If, on the other hand some point outside the sino-auricular node be rhythmically stimulated, flutter may be produced. If the juncture between the lower vena cava and the auricle, be chosen, the waves will spread up around the ring of vessels, through the sino-auricular node and on around to the starting point. They do not spread equally well in both directions, due probably to an unresponsiveness in certain portions of the muscular ring surrounding the veins. Here we are then dealing with a wave which circulates. It circulates around a natural ring of muscle formed by the orifices of the two venæ cavæ. About 380 circuits are completed in a minute. Flutter consists primarily of a continuous circulating wave. The path may be around the two veins, either clockwise or counter-clockwise. Only one cava may be encircled, or the circus movement may be around other natural orifices such as the mitral valve. The impulse originating in this circus movement spreads rapidly to all parts of the auricular tissue. Thus the movements of the entire auricle are controlled by the circulating wave. "There is the central or mother wave, and there are its centrifugal off-shoots into the remaining tissues." A single wave has thus circulated in certain known cases for as long as seven years.

The circus movement described depends for its maintenance upon three factors; first the length of the path traversed, second the rate at which the wave travels, and third, the duration of the effective refractory period.

"It must be evident that if the wave is to continue circulating a gap must exist between the crest of its advance and the wake of its retreat. As the crest travels it must always find the muscle which it enters in a responsive state. If for any reason the gap becomes closed the wave will proceed no longer and the rapid action of the auricle will cease. The three factors named, and these alone, control the length of the gap. The duration of the refractory period at any given point must always be less than the time spent by the traveling wave in completing its circuit; this circulation time depends on the length of the path and the rate of travel." Lewis has measured these three factors accurately in experimental cases. The gap between the crest of the wave's advance and the wake of its retreat is of extreme importance. Our ability to influence its length will determine our success in treating flutter. If by any means we can close the gap we will bring flutter to an abrupt termination.

The explanation of auricular fibrillation is based upon the same principle but the phenomenon is more complex. When the heart rate is increased, both the duration of the contraction and the refractory period become shortened.

The latter does not shorten proportionately. Consequently the responsive period diminishes when the rate is advanced. At a very high rate the responsive period disappears altogether. When this happens the muscle will not respond to every impulse reaching it and 2:1 block appears. As the rate of beating increases and the period of responsiveness shortens there develops what is spoken of as a partially refractory period. It is a phase of the cycle during which the muscle as a whole may or may not respond to stimulation. There are now three parts to the cycle following contraction; the absolutely refractory phase, the partially refractory period, and finally a short phase in which the muscle is entirely responsive to stimulation. In both fibrillation and flutter the gap between the crest and the wake consists not only of wholly responsive, but also of partially refractory muscle fibres.

"The oncoming crest encounters many fibres which are responsive; it finds some which are still refractory. These refractory fibres form minute barriers to its progress; it wends its way from side to side, passing only where it finds channels open and ready to receive it. It is as though a second prairie fire followed a first, but followed it at a time when the vegetation formerly burnt had not fully returned to its old condition; there would be places where the cinders of the first fire still blackened the earth, and the second flame in passing would creep around the edge of these before it could go forward. These barriers, by deflecting it, render the onrushing wave sinuous in its course, and delay its progress from point to point."

Fibrillation, like flutter, is maintained by a circulating wave or circus movement. Here again there is a single wave but the circuit is completed in a shorter time. The auricular cycles follow each other more rapidly. The path is shorter. This probably is because in auricles predisposed to fibrillation the effective refractory period is shorter than in those predisposed to flutter.

"The difference between fibrillation and flutter is in part one of rate; but that is not the sole difference or even the most important difference. It has been shown to be the rule that in flutter the crest of the circulating wave is constantly passing through tissue in a partially refractory state. The wave as it progresses strikes upon small barriers which render its course finely sinuous. In fibrillation this interference is much exaggerated; the barriers are larger and the crest travels along paths coarsely sinuous. Seemingly the crest has not a straight or simply curved border of advance; it is deeply and irregularly crenated. Its wake is similarly crenated, and the crenations of the one and the other overlap and intertwine. The crest of advance moves through muscle, apparently in a denser state of partial refractoriness than is the case in flutter."

In fibrillation as in flutter the same central path is covered again and again and the wave encircles the same type of central area but it does so in a "staggering" course. It is a road of many obstacles. Progress is therefore more difficult and less rapid, and the return to the starting point occurs after unequal intervals of time. For this reason and because of changes in the actual path pursued, the deflections in the electrocardiogram are of irregular

incidence and form. The paths through the outlying muscle vary. In each cycle the waves pass at least once through the auriculo-ventricular node, but because the path is coarsely sinuous and variable, waves arrive at this node at irregular intervals. The responses of the ventricle are therefore irregular.

Lewis points out that investigation of the refractory period of heart muscle is of extreme importance in the search for therapeutic remedies. Anything which will prolong the refractory phase will aid in closing the gap between the crest and the wake of the circulating wave, and thus will break the tendency to circus movement.

This pioneer work opens new and promising fields in cardiologic study.

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—W. T. V.

### *Intensive Treatment and Neurosyphilis*

IN a recent editorial the fact, discovered by Brown and Pearce, that modern antisyphilitic therapeutic measures lower the resistance of the individual to syphilitic reinfection was discussed. It will be remembered that these authors showed that animals were more susceptible to infection after they had received an injection of arsphenamine or neoarsphenamine, than they were if they received no treatment; in other words that, whereas the untreated animals were developing a greater or less immunity to reinfection, the treated ones reacted differently and showed no immunity.

Another aspect of this same problem, based upon extensive clinical rather than experimental observation is taken up by Fraser, whose thesis is that it is the duty of the syphilologist to conserve the antibody supply in order that fewer central nervous lesions be produced, unless he can assure himself that he has prevented a nervous system infection or unless he has killed off every individual spirochete. His argument is based upon the following statement: When the causal organism of syphilis gains entrance, a cell reaction occurs around the organism. A lesion may appear at the site of the inoculation after a certain period of incubation but even before this phenomenon is fully established, the stage of generalization has begun. The chancre is the real protective cell reaction, and the more vigorous this reaction, the longer is the generalization of spirochetes throughout the body delayed. As the so-called secondary stage appears, the reaction of the tissues against the general infection is demonstrated in the rash, or it may be, in a meningeal reaction. The entire destruction of the attacking organism by these reactions seldom takes place, and the organism is permitted to survive and increase. The central nervous system is apt to be invaded at the time of the general systemic invasion, and here, in the absence of a vigorous cell reaction, the spirochete may penetrate into relatively nonvascular structures where it may lead a harmless saprophytic existence for years. While the cell reaction is going on, antibody production is proceeding also, in proof of which is the resistance



to reinfection. The central nervous system, dependent upon the general systemic circulation for its antibody supply, suffers none so long as this supply is available. With this supply cut off the opportunity occurs for growth of the spirochetes and the development of active lesions. The object of treatment then should be, says Fraser, to conserve the antibody supply of the general system in order that it may act, not only generally but also locally in the nervous system. This he says is necessary because the blood circulatory system of the brain and cord is less available for lesions in those organs than in other parts of the body, and that therefore it is possible therapeutically to sterilize the body before the central nervous system is also sterilized. The accomplishment of general sterilization is generally accepted when the blood Wassermann reaction has become negative. At this point treatment is stopped, and thereafter the nervous lesions are left to develop unhindered and with only their locally produced antibodies to influence their development.

As Fraser puts it, "Modern treatment, on account of the tendency to work for a negative Wassermann, plays into the hands of the spirochete. By rapidly sterilizing the general systemic circulation it causes antibody production there to cease. It fails to sterilize the intrathecal system, but successfully cuts off its main source of antibody supply. The result, therefore, is that neurosyphilis occurs earlier and more frequently than was the case in pre-arsenobenzene days." Evidently, if this be true, the physician who wishes to sterilize quickly the patient should do so, and then when the blood Wassermann has become negative, should continue therapy, preferably mercurial, until he can feel assured that the central nervous system too has been rid of its spirochetes. From the laboratory standpoint this would mean when both blood and spinal fluid Wassermanns had become negative. The Army system of antiluetic therapeutics is based upon such a result. But with the laboratory results clinical observation must be combined, if the syphilologist be not "strangled by the tyranny of the Wassermann reaction." Beside the spinal Wassermann, and the spinal fluid lymphocytosis, the gold curve, etc., there are whole series of cases in which there are symptoms which Fraser groups as follows:

The *meningeal group*, in which headache, insomnia, somnambulance, noises in the head, vertigo, slight, severe or complete deafness, optic neuritis, diplopia and paralysis of the recti muscles and even opisthotonus are observed.

The *neurotrophic group* with acneiform and lichenoid syphilides, leucoderma, accompanied by meningeal symptoms and cerebrospinal fluid changes.

The *neurorecurrence group* with affections of individual cranial nerves, inequality of the pupils, sluggish reflexes and cardiovascular changes.

The *psychic group* with its melancholia, irritability, apathy, decreasing memory, moodiness, brutality and even suicidal tendencies.

Largest of all is the *asymptomatic group* in which the spinal fluid usually, but not always, shows suggestive changes.

By systematic study of the clinical phenomena one may judge better than by mere serologic work where a patient stands with respect to his infection, and to a certain extent at least "the method of wholesale arsenobenzene given rigidly and blindly until the Wassermann reaction is negative" can be abandoned. At

the same time a clearer notion of what syphilis may do will be obtained by the physician.

Fraser insists upon the inclusion of *intramine* in any system of treatment of syphilis. This drug he says exerts a protective action upon the nervous tissues. Of it McDonagh says that much nervous syphilis can be prevented by the routine use of intramine which prevents arsenic and mercury from exercising a toxic influence on nerve tissue. It has been used by Ffrench in the treatment of icterus and exfoliative dermatitis occurring during syphilitic treatments.

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—P. G. W.

### *Medical and Health Conditions in Soviet Russia*

THERE has recently appeared in the Lancet, London, (April 23, 1921) an article by Dr. Boris Sokolov, Professor of Protozoölogy at the University of Petrograd, in which he depicts the actual state of medical practice and health in Russia. The description is matter-of-fact, but through the lines one may read an infinitude of misery, anguish and suffering.

The Soviet medical organization is based upon rigid centralization, under the control of the Department of Public Sanitation and Hygiene, which directs the entire medical work of the country. The development of a bureaucracy has resulted in an extreme laxity in administration. Directors of the hospitals and clinics are members of the Communist Party and usually are political appointees of little or no ability, but having absolute, dictatorial power.

Scientific work is practically at a standstill. Only a very few medical journals have survived and the laboratories have neither money nor equipment for experimental work. What little research is being carried on has been chiefly in the study of typhus fever.

The Soviet government appears to have at last assumed a tolerant attitude towards the medical profession. Members of this profession are the only "intellectuals" whose work has not been entirely interrupted by the Communist Revolution. The medical men have attempted to maintain throughout a neutral attitude in political matters. As a body they are however, anticommunist. As a result of this neutrality many have been shot by the Reds, and many others by the Whites. Many physicians are still in prison. The doctors have felt it their duty to maintain in so far as possible, the health of the masses, the majority of whom cannot be held responsible for the Revolution or its consequences. Twenty-five per cent of the profession have left Soviet Russia.

Physicians have suffered more from epidemic disease than from persecution or civil war. Between November, 1917, and August, 1920, 47 per cent of the medical men in Petrograd died. Sokolov remarks that it would be difficult to find a single doctor or nurse who had not suffered from two or three attacks of continued fever of some form or other. In the spring of 1920 a gen-

eral improvement in health was noticed. It has been suggested that by this time practically the whole population had been exposed to or infected with the various infectious diseases and that the entire population was practically immune. Within six months' time the percentage of persons suffering from epidemic diseases decreased from thirty per cent to seven per cent.

The economic situation is acute. When the Soviet power was first established equalization of wages was instituted, and experienced doctors earned little more than their assistants. As a result many gave up practice and attempted to earn a living along other lines. Later, however, they were recruited by the Government, and given better salaries. Even now the salaries are preposterously low. Assistants to doctors are paid 4,000 roubles per month. A pound of butter costs 5,000 roubles. The bourgeois clientele of pre-revolutionary days can no longer afford the services of the physician. The paying patients now come from the Communist bureaucracy, or are successful speculators. Professor Ov recently charged 100,000 roubles for a small operation performed upon the wife of a leading Communist. This sum is not at all extraordinary. The number of private patients that a physician may have is limited and no doctor may write more than fifty prescriptions for private patients, each month.

The condition in the hospitals is improving, but even now the staff discipline is lax, and the sick are often ill-treated, while delirious patients have been beaten. The doctors do not have full control and can do little to stop this abuse. The central infirmary at Moscow was found in August, 1920, to be in a state of chaos. There was no isolation, and diagnosis was but superficially made. Cases of typhoid and tuberculosis were to be found in the same ward. There was a shortage of medicine and of surgical apparatus throughout Russia. The hospitals in Petrograd and Moscow still had quinine, aspirin and anesthetics, but the reserve stock was nearly depleted. Only a few kilograms of chloroform remained in Petrograd, and all of the morphine had been used up. A salvarsan preparation was being sold, but it was of bad quality. Surgical instruments were running short.

During the last three years there have been 31,789,230 reported cases of infectious disease in Soviet Russia.

Some diseases have disappeared, while new ones have made their appearance. Among the former are obesity and chronic catarrhs. Diseases common among the middle class, brought about by idleness have to a great extent disappeared. A Petrograd physician states that out of 300 patients only nine were found to be suffering from fatty degeneration of the heart and of these, five were well-known Communists and the other four were profiteers. A new illness has recently appeared, due to the eating of badly cooked wheat. The symptoms consist of pain in the right side of the abdomen, dizziness, nausea and distaste for food. Tuberculosis is spreading rapidly and widely. Bad food had brought about a general exhaustion and various forms of anemia. The general ill health is the most striking phenomenon to any one newly arrived in Russia. Professor Ov states that 30 per cent of women and girls are no longer menstruating because of anemia and undernourishment. A large

increase in miscarriages has resulted. Women are terrified at the thought of bearing a child, knowing full well that if born alive the infant will shortly die. The government has abolished open prostitution and has regulated brothels, but there are more prostitutes today than at any time previously.

Sokolov remarks that the greatest danger of recent Russian history is apparent in the younger generation. "Parents are overworked and unable to bring up their children properly; the lack of control is having the most serious results; the actual destruction wrought by the revolution is nothing to the appalling moral degeneracy and criminality among children, which now stands out as the foremost evil of modern Russia. At one hospital, where the number of children suffering from venereal disease used never to be higher than 15 per cent, 60 per cent are now affected as compared with 40 per cent of adults." The children are weak and ailing on account of undernourishment. The new generation is being overcome by eczema, anemia and rickets. The government is now considering nationalization of the children.

Dimitri Mereshkovski has written, in the *Deutsche Allgemeine Zeitung*, "The Bolsheviki preach the destruction of the old civilization in order that a new civilization may be erected in its place. But their words are folly, deception, ignorance; their acts are all that count. We must give them credit that they understand how to destroy. The world has never before seen such destroyers.

"Bolshevism is a reversion to nature; but those who have gone wild in civilized society are powerfully attracted to a state of primitive nature. Europe having reverted to savagery, is drawn toward savage Russia.

"Bolshevism is barbarism, but wearied civilization longs for barbarism as a stifling man longs for air.

"Bolshevism is brutalization. But we must remember that Voltaire said: 'When I read Rousseau, I want to run about in the woods on all fours.' All Europe, when it watches the Bolsheviki, conceives a similar desire to course the forests like the beasts.

"Bolshevism is nakedness; but Europe says, like the dead man in Dostoevski's *Bobok*: 'We, too, will be naked.'

"Bolshevism is the pest, but Europe has already become *A Banquet During the Pest*.

"Bolshevism is the end of the world; but the world wants to end.

"Bolshevism is the suicide of Europe. Tolstoy inspired the act; Lenin completed it."

—W. T. V.



# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, FEBRUARY, 1922

No. 5

## *ORIGINAL ARTICLES*

### THE INTERNAL SECRETION OF THE PANCREAS\*

BY F. G. BANTING, M.B., AND C. H. BEST, B.A.

THE hypothesis underlying this series of experiments was first formulated by one of us in November, 1920,<sup>†</sup> while reading an article dealing with the relation of the isles of Langerhans to diabetes.<sup>1</sup> From the passage in this article, which gives a résumé of degenerative changes in the acini of the pancreas following ligation of the ducts, the idea presented itself that since the acinous, but not the islet tissue, degenerates after this operation, advantage might be taken of this fact to prepare an active extract of islet tissue. The subsidiary hypothesis was that trypsinogen or its derivatives was antagonistic to the internal secretion of the gland. The failures of other investigators in this much-worked field were thus accounted for.

The feasibility of the hypothesis having been recognized by Professor J. J. R. Macleod, work was begun, under his direction, in May, 1921, in the Physiological Laboratory of the University of Toronto.

In this paper no attempt is made to give a complete review of the literature. A short résumé, however, of some of the outstanding articles which tend to attribute to the isles of Langerhans the control of carbohydrate metabolism, is submitted.

In 1889 Mering and Minkowski<sup>2</sup> found that total pancreatectomy in dogs resulted in severe and fatal diabetes. Following this, many different observers experimented with animals of various species and found in all types examined, a glycosuria and fatal cachexia after this operation. The fact was thus established that the pancreas was responsible for this form of diabetes. In 1884, Arnozan and Vaillard<sup>3</sup> had ligated the pancreatic ducts in rabbits and found that within twenty-four hours the ducts become dilated; the epithelial cells begin to desquamate; and that there are protoplasmic changes in the acinous cells. On the seventh day there is a beginning of

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round-celled infiltration. On the fourteenth day the parenchyma was mostly replaced by fibrous tissue. Sseobolew<sup>4</sup> in 1902 noted in addition to the above, that there was a gradual atrophy and sclerosis of the pancreas with no glucosuria. However, in the later stages, from thirty to one hundred and twenty days after ligation of the ducts, he found involvement of the islets and accompanying glucosuria.

Lewaschew<sup>5</sup> believed that the islets were modified acinous cells. Laguesse,<sup>6</sup> an anatomist, first suggested that the islets might be the organ of pancretic internal secretion. He showed that there were comparatively more islets in the fetus and the newborn than in the adult animal. Opie<sup>7</sup> and Sseobolew<sup>8</sup> independently furnished the first clinical foundation for the belief that the islets were involved in pancretic diabetes.

W. G. MacCallum,<sup>9</sup> in 1909, ligated the ducts draining the tail third of the pancreas. After seven months he excised the remaining two-thirds. This was followed by a mild glucosuria. Three weeks later he removed the degenerated tail third. This second operation resulted in an extreme and fatal glucosuria. Kirkbridge,<sup>10</sup> in 1912, repeated and corroborated MacCallum's findings and, by the use of Lane's<sup>11</sup> method of staining, proved that the atrophic tissue contained healthy islets.

Kamimura<sup>12</sup> in 1917, working on rabbits, traced the degenerative changes in the parenchymatous tissue of the pancreas after ligation of the ducts, and found that the islets remained normal and that the animal did not develop glucosuria as long as the islets were left intact.

The first attempt to utilize the pancreas in defects of carbohydrate metabolism was made by Minkowski.<sup>13</sup> This worker tried the effect of pancretic feeding, with no beneficial results. Up to the present time only useless or even harmful effects have been obtained from repeated attempts to use this method.

Knowlton and Starling,<sup>14</sup> in 1912, published experiments which showed a marked decrease in the power of using sugar of a diabetic heart perfused outside the body, as compared with a normal heart under similar conditions. Macleod and Pearee,<sup>15</sup> using eviscerated animals were unable to confirm the above results. Patterson and Starling<sup>16</sup> subsequently pointed out that a serious error was involved in the early experiments due to (1) excess glycogen present in diabetic hearts, and (2) to the irregular disappearance of glucose from the lungs.

Murlin<sup>17</sup> prepared an alkaline extract of pancretic tissue and after injection of this solution, secured a reduction in sugar excreted in a diabetic animal. Kleiner<sup>18</sup> has pointed out that the reduction secured by Murlin might be due to the alkali *per se*. Kleiner himself has shown that "unfiltered-water extracts of fresh pancreas diluted with .90 per cent NaCl when administered slowly usually resulted in a marked decrease in blood sugar." There was no compensating increase in urine sugar, but rather a decrease, which Kleiner suggests may be partly due to a temporary toxic renal effect. Hemoglobin estimations made during the experiment showed that the reduction in blood sugar was not a dilution phenomenon. Paalesen<sup>19</sup> has recently demonstrated

the reducing effect of whole gland extract upon the amounts of sugar, urea and acetone bodies in the blood and urine of diabetic animals. He states that injections into peripheral veins produce no effect and his experiments show that second injections do not produce such marked effect as the first.

From the work of the above-mentioned observers we may conclude: (1) that the secretion produced by the acinous cells of the pancreas are in no way connected with carbohydrate utilization; (2) that all injections of whole-gland extract have been futile as a therapeutic measure in defects of carbohydrate utilization; (3) that the islands of Langerhans are essential in the control of carbohydrate metabolism. According to Macleod there are two possible mechanisms by which the islets might accomplish this control: (1) the blood might be modified while passing through the islet tissue, i.e., the islands might be detoxicating stations and (2) the islets might produce an internal secretion.

We submit the following experiments which we believe give convincing evidence that it is this latter mechanism which is in operation.

In the ten-week interval which we considered necessary for complete degeneration of the acinous tissue, we secured records of dogs depancreatized by the Hédon method.<sup>20</sup>

#### METHODS

The first chart is a record of an animal depancreatized by the Hédon method. The details of this operation are given in Hédon's article.<sup>20</sup> The remaining records are of animals (females) completely depancreatized at the initial operation. The procedure is as follows: under general anesthesia an upper right rectus incision is made through the abdominal wall. The duodenum is delivered through the abdominal wound, and the pancreas traced to the tail portion. The mesentery beyond is cut between clamp and ligature. Vessels from spleen are then isolated, ligated and divided. Little dissection is then required until the duodenum is reached. The superior pancreaticoduodenal vessels are located and great care is exercised to avoid damaging them. The pancreas is stripped from the duodenum by dry dissection. The vessels to the uncinate process are ligated and divided, and the process freed from its mesenteric attachments. The larger duct of the pancreas is then ligated close to its entry into the duodenum and the pancreas is removed. Special care must be exercised to preserve the splenic vessels. The superior pancreaticoduodenal vessels must be left intact. Failing this, duodenal ulcer is a frequent development. If this procedure is carried out the whole gland with the exception of the portion in contact with the duodenum is covered with mesentery. The abdominal wound is closed layer by layer with catgut. A collodion dressing is used. The urethral orifice is exposed by a midline incision of the perineum and the edges of the wound drawn together to facilitate healing.

We have found that animals between eight and sixteen months old are the most suitable for this operation. At this age the pancreas is not so firmly fixed as it becomes later.

We first ligated, under general anesthesia, the pancreatic ducts in a



number of dogs. (Blood sugar estimations on these animals were recorded from time to time. We have no record of a hyperglycemia).

The extract was prepared as follows: The dog was given a lethal dose of chloroform. The degenerated pancreas was swiftly removed and sliced into a chilled mortar containing Ringer's solution. The mortar was placed in freezing mixture and the contents partially frozen. The half frozen gland was then completely macerated. The solution was filtered through paper and the filtrate, having been raised to body temperature, was injected intravenously.

We have never found it necessary to cut down on a vein under general or local anesthetic. The skin surface above the vein is shaved and the needle inserted into the vein which is dilated by compression. The dogs make very little resistance to this procedure and after the first few punctures lie quietly during the operation. Sugar injections (100 c.c. of fluid) as well as the numerous administrations of extract were conducted by this method.

We performed several experiments with the object of exhausting the zymogen granules of the pancreas. Prolonged secretin injections and vagus stimulation below the diaphragm were practiced. Fortune favored us in the first experiment. In subsequent attempts we were never able to exhaust the gland sufficiently to obtain an extract free from the disturbing effects of some constituent of pancreatic juice.

The blood sugar estimations were made by the Myers-Bailey<sup>21</sup> modification of the Lewis-Benedict method. The results of this method were corroborated by the Schaffer-Hartman<sup>22</sup> method at high and low percentages of blood sugar. The former method gave results which were consistently slightly higher (.01 per cent) than those obtained by the Schaffer-Hartman method. We find the average normal blood sugar, from observations on thirty normal dogs, to be .090 per cent.

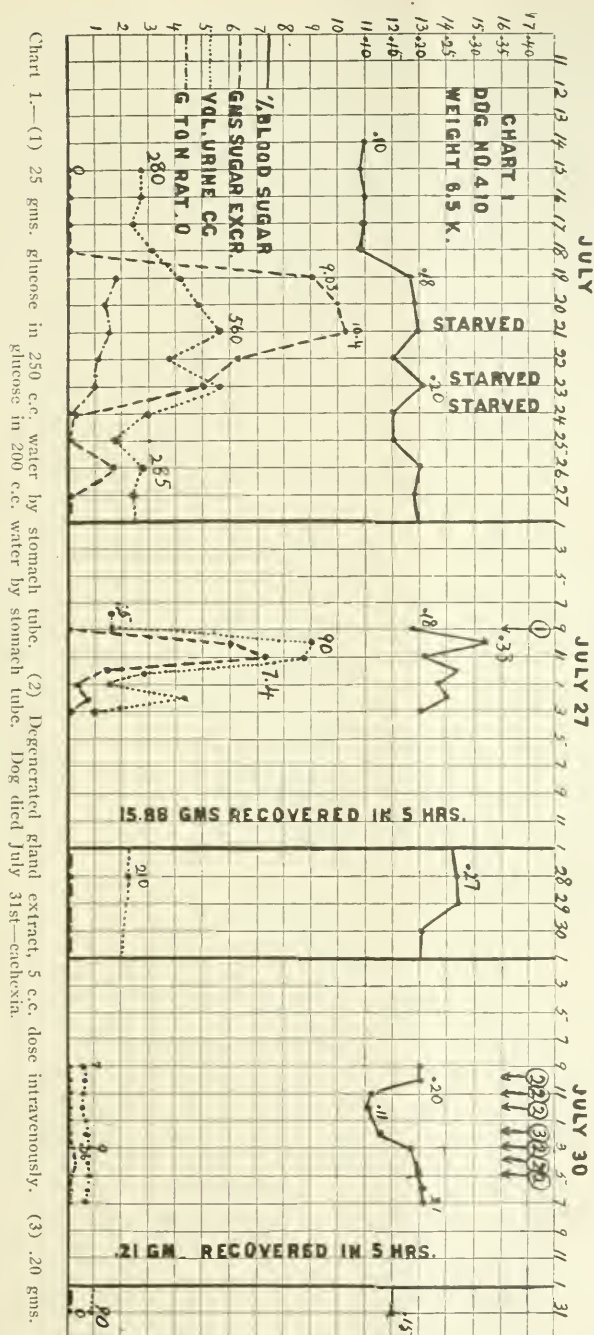
Hemoglobin estimations were made by the carbon-monoxide saturation method, using the du Boscq colorimeter.

#### RESULTS

Chart 1 contains the record of a 6.5 kilogram dog (410). This experiment is not conclusive but is interesting to us at least, since we administered the first dose of extract of degenerated pancreas to this animal. On July 11, the pancreas, with the exception of the processus uncinatus, was removed. The processus was allowed to remain until July 18. In the interval between the operations there was no hyperglycemia or glucosuria. The curves on subsequent days show the effect produced by the removal of the pedicle. It will be noted that as the experiment progresses the percentage of blood sugar did not rise to the level usually attained in completely depancreatized animals, and also that there was a marked decrease in the daily amounts of nitrogen and sugar excreted and the volume of urine voided. The animal continued to lose weight and seemed to be entering the cachexial condition characteristic of depancreatized animals which had become infected.

The chart for July 27 shows the effect produced on the percentage of





blood sugar and on the sugar excretion by the oral administration of twenty-five grams of dextrose in two hundred and fifty c.c. of water.

At 10 A.M. July 30, the percentage of blood sugar was 0.20. Four c.c. of extract of degenerated pancreas were injected intravenously. At 11 A.M. the blood sugar had fallen to 0.12 per cent. The injections of extract are shown

in the chart. At 12 A.M. twenty grams of sugar in two hundred c.c. of water were given by stomach tube. The chart records the effect.

The obvious criticism of this experiment is that the animal was moribund when the effect of the extract was tried. The interesting features, which gave us great encouragement are (1) the extract caused a sudden fall in the blood sugar and (2) that in the presence of the extract the animal excreted .21 grams of a 20 gm. injection in a period of five hours following the injection, in contrast to an excretion of 15.88 grams of a 25 gm. injection in the same interval, when no extract was administered.

Chart 2 is the record of dog 92, weight 11.9 kg. A complete pancreatectomy was performed on this animal at three P.M. August 11. The first injection of extract was given six hours after the operation and subsequently an injection every four hours. This extract was freshly prepared from a ten Kg. dog whose pancreatic ducts had been ligated for ten weeks. One hundred and twenty-five c.c. of extract were prepared from the gland residue but this supply was exhausted by two P.M. August 13, after which other extracts were used. Blood samples were always taken before the injections of extract.

On August 12, the blood sugar curve shows that neither five nor eight c.c. of this extract every four hours were sufficient to counterbalance the upward trend of the percentage of sugar of the blood. At 10 P. M. the dose was increased to twelve c.c. and a marked fall is noted. The chart at 10 A. M. August 14 records the reduction of the percentage of sugar in the blood below its normal level, as a result of extract from another degenerated gland. (The exceptionally higher values for the volume of urine and the urinary nitrogen for August 15 and 16 may be due to the adulteration of the urine with vomit.) On August 15 at 10 A. M. the chart shows the effect produced by ten c.c. of the same gland extract made 0.1 per cent acid with HCl. This extract made 0.1 per cent alkaline with NaOH causes a slight reduction (August 15, 8 P. M.) The effect may be due to the alkali.

The extract administered at 10 A. M. August 16 was neutral and made from the same degenerated gland.

On August 16 and 17 effects of extracts from normal glands were tested. A normal pancreas from a ten kg. animal was divided into three equal parts. One third was extracted with neutral saline, the second portion with 0.1 per cent HCl and the third with 0.1 per cent NaOH. On August 17 at four P. M. the neutral whole gland extract was administered. A marked fall in blood sugar resulted. The acid and alkaline extracts were injected at 12 P. M. August 17, and 7 A. M. August 18. The last two injections were perhaps not given a fair opportunity to develop their effects. We do not take colorimeter readings by artificial light and therefore did not have an accurate knowledge of the height of blood sugar at these times.

The conclusion from this experiment is that freshly prepared neutral or acid extracts of the whole pancreas do have a reducing effect on blood sugar, thus confirming Kleiner. It may be stated here that repeated injections of whole gland extracts cause marked thrombosis of the veins where the injections are made and a noticeable interference with kidney function. It is obvious from the chart that the whole gland extract is much weaker than that from the degenerated gland.

On August 20, we attempted to exhaust the pancreas of a nineteen kg. dog by continued injections of secretin and repeated stimulation of the vagus nerve below the diaphragm. We obtained eighty-five c.c. of pancreatic juice and considered the gland exhausted. It was swiftly removed and immediately chilled. The marked effect of injection of this material is shown on the chart at 7 P. M. August 20. On August 21 we incubated ten c.c. of the extract and five c.c. of pancreatic juice for two hours at

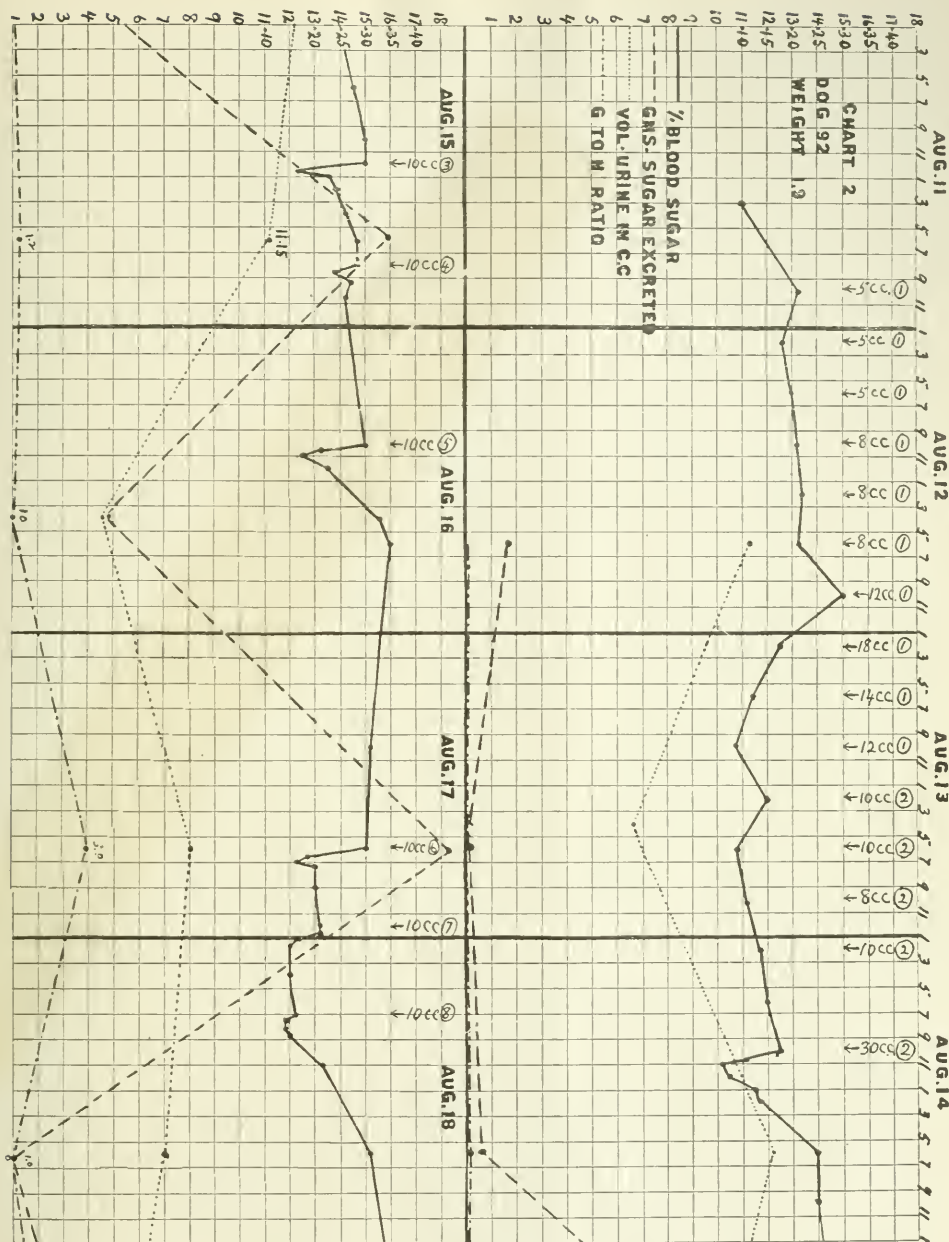


Chart 2.—(1) Degenerated pancreas, dog 394. (2) Degenerated pancreas, dog 390. (3) Degenerated pancreas, + .1% HCl. (4) Degenerated pancreas + 0.10% NaOH. (5) Degenerated pancreas, + .1% HCl. (6) Whole gland extract, fresh, cold. (7) Whole gland extract, + .1% HCl. (8) Whole gland extract + .1% NaOH. (9) Exhausted gland extract. (10) 10 c.c. exhausted gland extract + 5 c.c. pancreatic juice incubated 2 hours. (11) 10 c.c. exhausted gland extract (—pancreatic juice) incubated 2 hours. (12) Whole gland, cat. Dog died August 30.

body temperature in alkaline solution. This solution was injected at 6 P. M. August 21. The curve shows the very slight effect produced. As a control on the above, ten c.c. of extract and five c.c. of saline were incubated under similar conditions for two hours. The chart at 10 P. M. August 21 records the marked effect of the injection of this second solution. On August 22 at 6 P. M. eight c.c. of extract from the normal



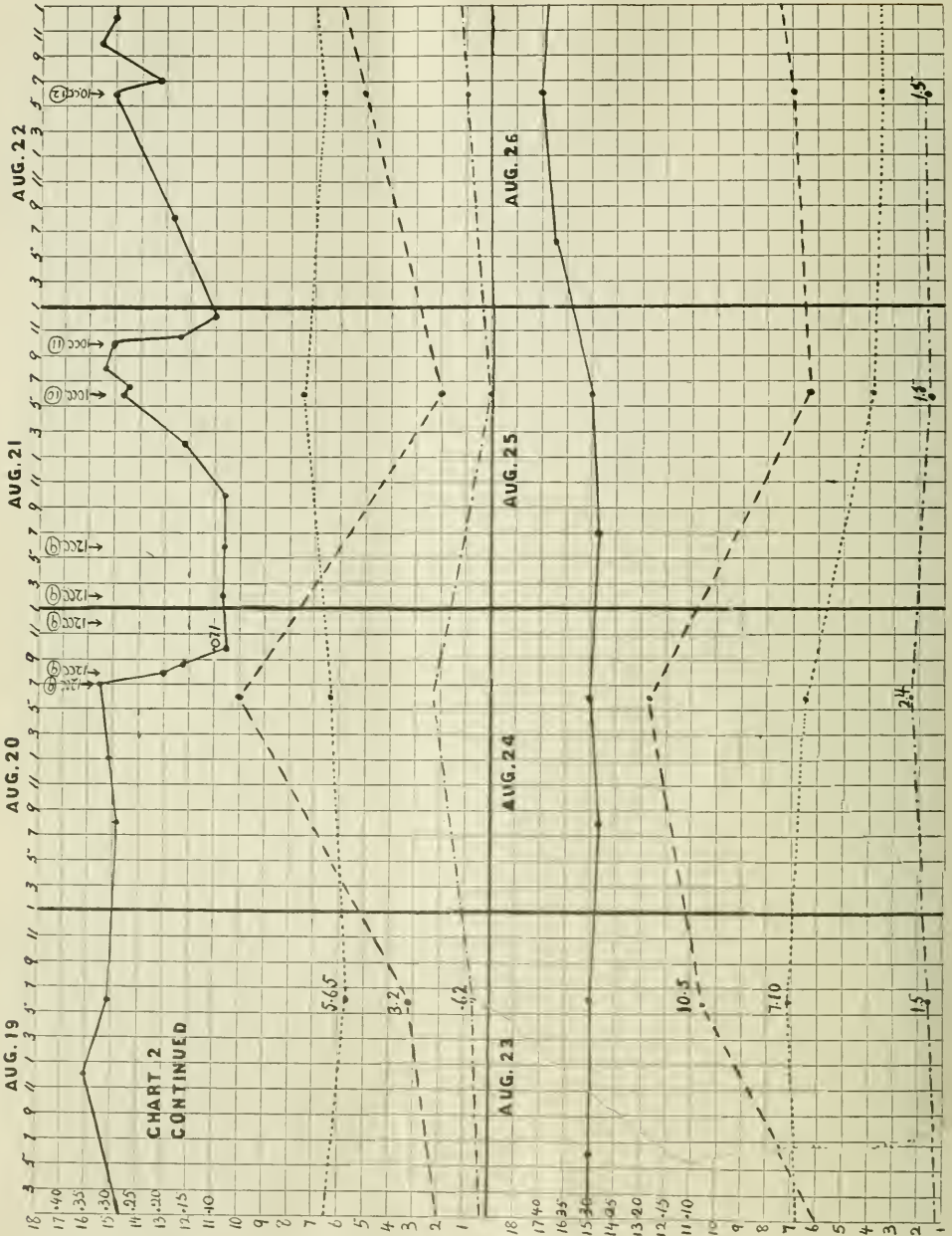


Chart 2.—Continued

pancreas of a cat were injected. We obtained a marked anaphylactic-like reaction. The curve shows the effect upon the blood sugar.

No further injections were given to this animal after August 22. The dog died on August 30, nineteen days after the operation. The autopsy showed consolidation and necrosis of a large area in lower lobe of right lung, infection in right pleural sac. The operation wound was well healed. There was no sign of pancreatic tissue. The abdomen was not infected.



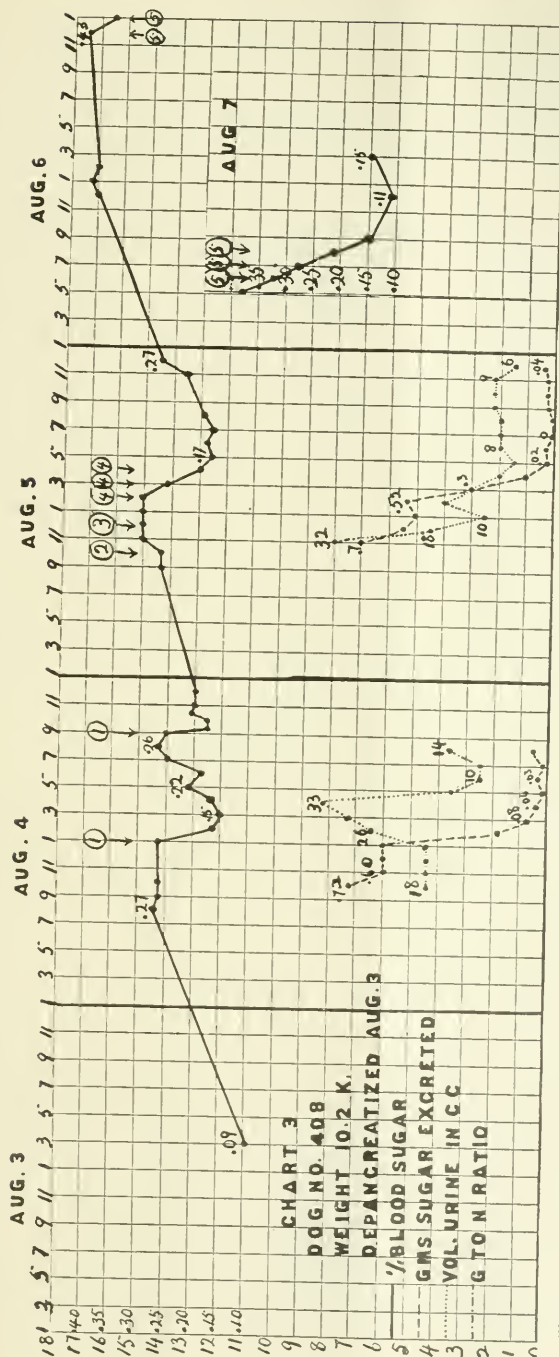


Chart 3.—(1) 5 c.c. 4 day old extract of degenerated pancreas. (2) 5 c.c. extract of liver. (3) 5 c.c. extract of spleen. (4) 5 c.c. extract of degenerated pancreas. Dog died August 7—general peritonitis.

Chart 3 is the record of dog 408. The weight of this animal was 9 kg. The details of the experiment will be given rather fully.

The normal blood sugar of dog 408 was .090 per cent. Eighteen hours after pancreatectomy the percentage of sugar in the blood was .27. Twenty-two hours after

the operation, 1 P. M. August 4, the blood sugar was .26 per cent. During the twenty-two hours 3.10 grams of sugar were excreted. The volume of urine was 494 c.c. At one P. M. we administered five c.c. of extract of degenerated pancreas which had been prepared four days previously and kept in cold storage. At two P. M. the blood sugar was .16 per cent. At three P. M. the percentage of sugar in the blood had fallen to .15. From one to three P. M. .19 grams of sugar were excreted in a volume of twenty-

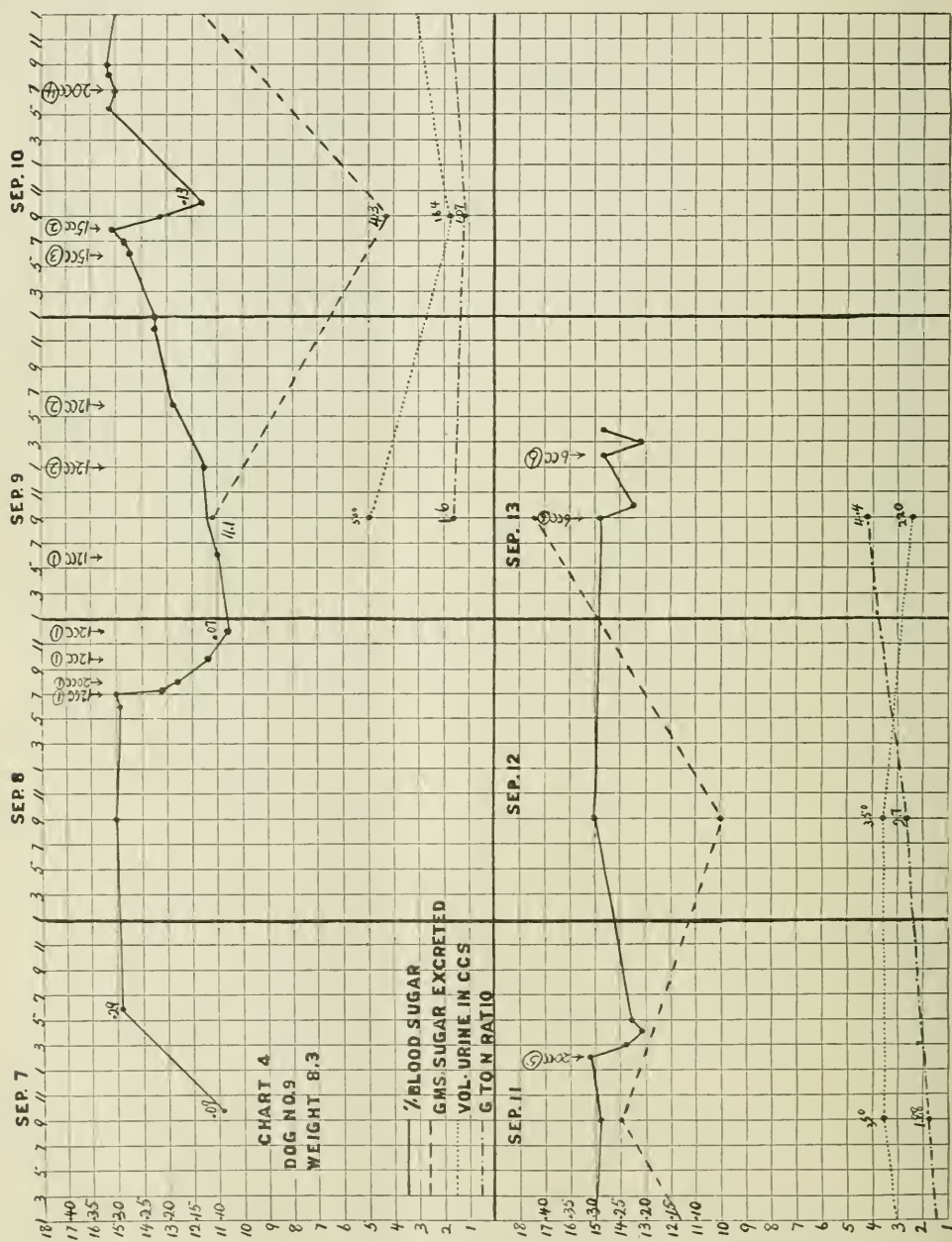


Chart 4.—(1) Exhausted gland extract 1% HCl. (2) Exhausted gland extract neutralized. (3) Exhausted gland extract neutralized per rectum. (4) 20 c.c. exhausted gland extract and alkali + 10 c.c. pancreatic juice incubated 3 hours at 37°C., then neutralized. (5) 20 c.c. exhausted gland extract incubated 3 hours at 37°C., 0.1% HCl. (6) Exhausted gland of cat, 0.2% HCl.

six c.c. of urine. Three and forty-three hundredths grams of urinary nitrogen were excreted in the twenty-four hours following the operation. The G. to N. ratio for this period was 1.4:1. From 3 P. M. to 7 P. M. the percentage of sugar in the blood shows a gradual rise from .15 to .25 per cent. This latter level was maintained until 9 P. M. The chart shows a slight rise in sugar excretion following the rise of blood sugar. At 9 P. M. five c.c. of extract which had been exposed to room temperature for one hour was injected intravenously. The blood sugar was reduced to a value of .18 per cent. The chart shows a gradual ascent from this value to .27 per cent. At 10 P. M. the percentage of sugar in the blood was .27. At this hour five c.c. of extract of liver, prepared in precisely the same manner as the pancreatic extract, were administered intravenously. One hour later the blood sugar was .30 per cent. This level was maintained during the following three hours. It was unaffected by an injection of 5 c.c. of extract of spleen. The chart shows the rise in volume of urine and amount of sugar excreted. At 2 P. M. (b. s. .3 per cent), five c.c. of an extract of degenerated pancreas were injected. A sharp fall in the blood sugar resulted. At 3 P. M. and again at 4 P. M. a similar dose of extract was given. The chart records the lasting effect. The 2 P. M. level of .30 per cent was regained twelve hours after the first injection. The hourly excretion of sugar ran approximately parallel with the percentage of sugar in the blood. Between 1 P. M. and 2 P. M. .52 grams were excreted. Less than .02 grams were excreted between 7 P. M. and 8 P. M. The highest glucose to nitrogen ratio observed in this experiment was a 3:1 value for the 22-hour interval between 2 P. M. the 6th of August and noon the following day. At 12 noon August 6 the percentage sugar in the blood was .40 per cent. Five c.c. of boiled extract of degenerated pancreas was injected intravenously at this stage and caused no reduction of blood sugar. At twelve midnight August 6 five c.c. of extract of degenerated pancreas which had been prepared 48 hrs. previously were administered. The blood sugar fell from .43 per cent at 12 P. M. to .37 per cent at 1 A. M. Five c.c. doses were given at 1, 2 and 3 A. M. and a twenty-five c.c. dose at 4 A. M. The chart shows the reduction in blood sugar to a normal level and the beginning of an upward trend five hours after the last injection of extract. The animal died at 12 A. M. August 7.

A brief description of the clinical condition of the animal at various stages of the experiment is necessary for the correct interpretation of the above results. The animal made a good postoperative recovery and was able to retain water and meat after the second day following the operation. On the morning of August 5 we noticed that the condition of the animal was much worse. It appeared excessively tired, did not eat, and vomited after drinking water and also after extract of spleen given intravenously. At 5 P. M. August 5 the animal appeared considerably improved. It retained water and ate meat. On August 6 at 10 P. M. the abdominal wound was moist with exudate, and the animal was not so active as on the preceding day. No marked variation from this condition was observed until 4 A. M. when 25 c.c. of extract were administered. After this injection the animal had a marked reaction and appeared to be dying. It was revived slightly by intravenous and intraperitoneal injections of warm saline. Considerable improvement was noted at 7 A. M., dog was able to stand. The improvement was short-lived. The dog died at 12 A. M. August 7. The post-mortem showed a widespread abdominal infection. There was no sign of pancreatic tissue.

The entire degenerated pancreas from one 8 kg. dog and approximately one-half the degenerated gland from a 6 kg. dog was the substrate of the extract used in this experiment.

Chart 4, Dog 9, gives additional evidence on several important points which have been referred to previously. At 6 P. M., September 8, we administered ten c.c. of extract of degenerated pancreas *per rectum*. There was no reduction in blood sugar at 7 P. M. when we gave 12 c.c. of extract of exhausted gland intravenously. The chart records the effect of this and sub-



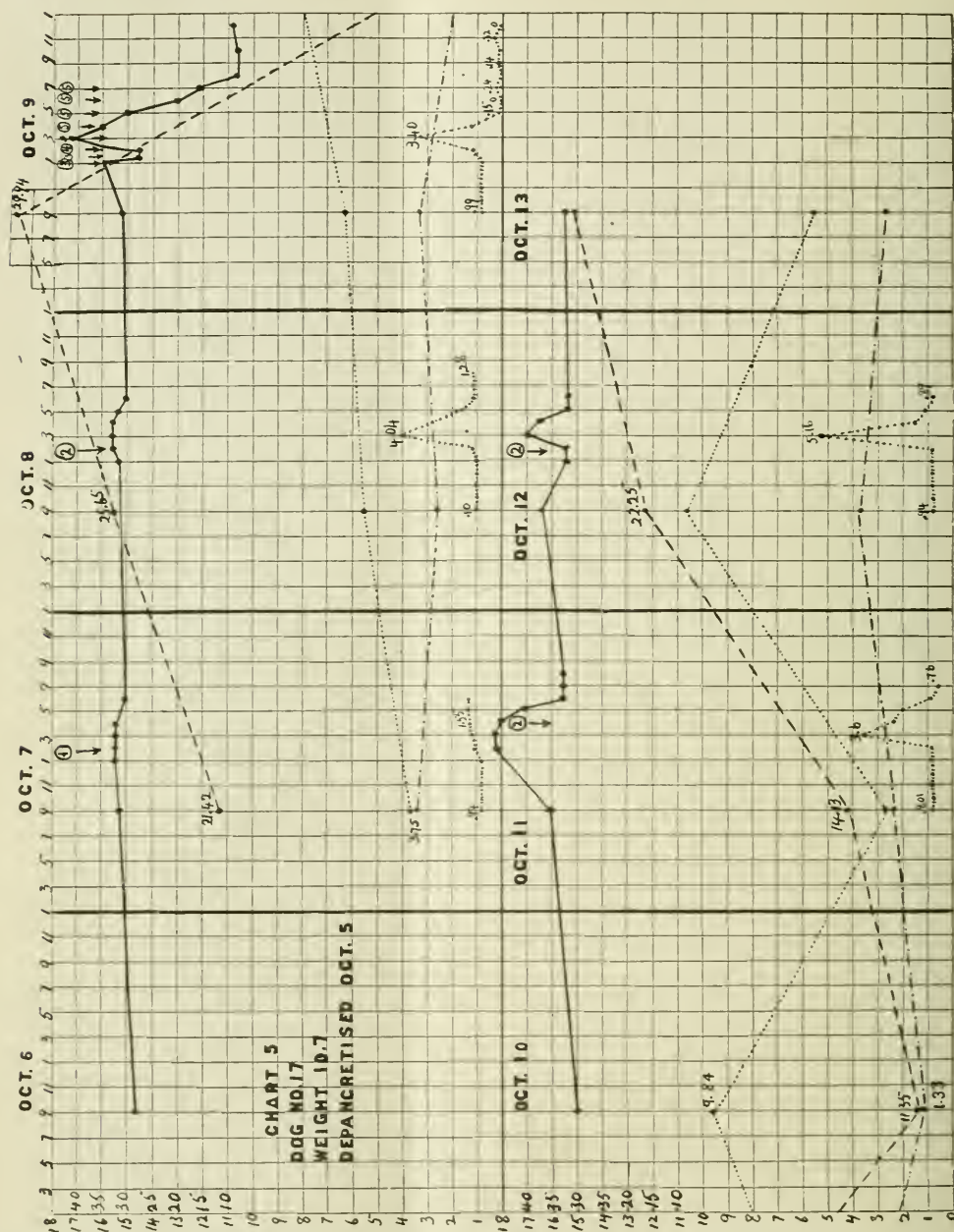


Chart 5.—(1) 100 c.c. saline. (2) 10 gms. sugar + 100 c.c. water. (3) 10 c.c. extract A. (4) 10 gms. sugar, 80 c.c. water, 20 c.c. extract A. (5) 20 c.c. extract B. *Note:* Extract A was made from uncinatæ process. Extract B from tail portion of pancreas

sequent injections of the same material. At 6 A.M., September 10, we administered 15 c.c. of extract of exhausted gland per rectum. There was no effect. At 8 A.M., September 10, fifteen c.c. of extract of exhausted gland were injected intravenously. The drop in blood sugar was very marked. Twenty c.c. of exhausted gland extract, made 1 per cent alkaline with NaOH, were



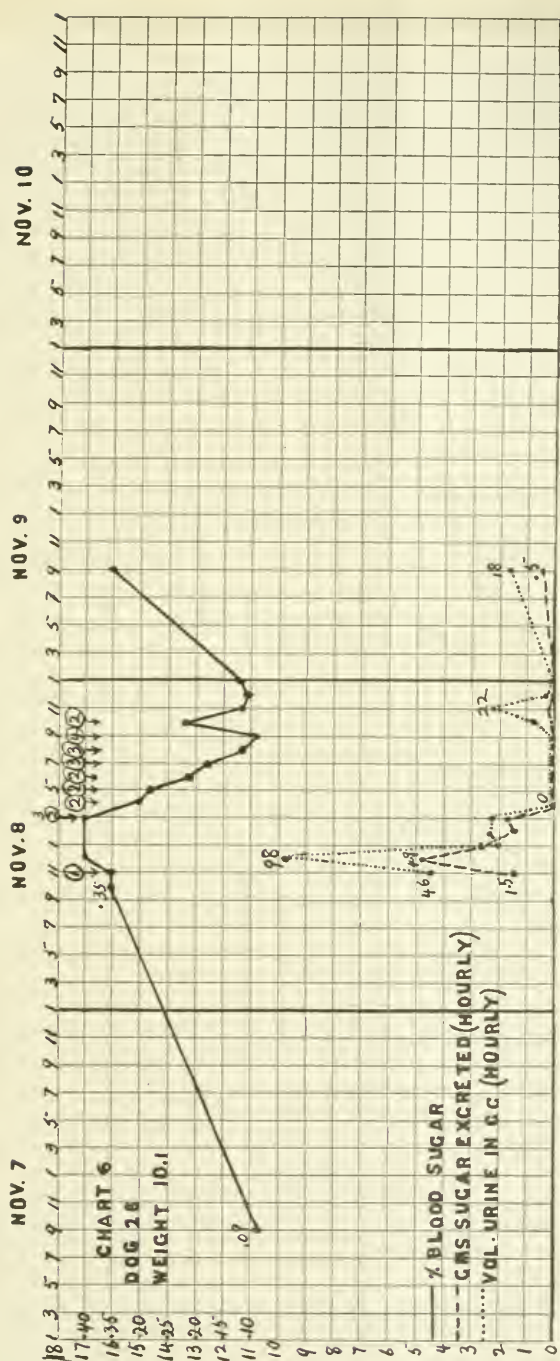


Chart 6.—(1) 10 gms. sugar in 100 c.c. water. (2) 15 c.c. extract degenerated pancreas (4 weeks after ligation of ducts). (3) 6 c.c. extract degenerated pancreas (7 weeks after ligation of ducts). (4) 10 gms. sugar in 100 c.c. water. Dog died November 10—duodenal ulcer.

incubated three hours at body temperature with 10 c.c. of active pancreatic juice. This solution was neutralized and injected intravenously at 7 P.M. September 10. No reduction in blood sugar resulted. At 2 P.M. September 11, 20 c.c. of acid extract incubated for three hours at 37.5° F. were injected. The curve shows the drop in blood sugar. On September 13 at 9 A.M. and

2 P.M. the effect of extracts from the partially exhausted gland of a cat is shown. This extract produces a pronounced general reaction.

We observe that extracts prepared from these more or less exhausted glands, while retaining to some extent the reducing effect upon blood and urine sugar, produce many symptoms of toxicity which are absent after injections of extracts from completely degenerated glands.

Chart 5 is the graphic record of an experiment on a 10 Kg. dog in which we have attempted to prove that the reduction of the percentage sugar in the blood is not a dilution phenomenon. Our plan of campaign was to inject at a given hour on the first day (2 P.M., October 7), 100 c.c. of isotonic saline (1). On the second day at the same hour the animal received 100 c.c. of 10 per cent sugar solution (2). Extract (3) (10 and 15 c.c. doses) was given one hour and a second dose thirty minutes before the corresponding hour on the third day and 70 c.c. of distilled water containing 10 gms. of sugar were injected on the hour (4). Extract in 20 c.c. doses was injected at 3, 4, 5, 6 and 7 P.M. (5). To interpret correctly the curve for this day, a brief description of extracts (3) and (5) is necessary. The pancreas from which these extracts were made was not completely degenerated. The pancreatic ducts of the animal had been tied six weeks. Extract (3) was made from the processus uncinatus. Extract (5) was made from the remainder of the gland. The extract from the uncinatus process was much weaker than the latter. In the four hours following the first injection of sugar we recovered 9.94 grams in the urine. In the corresponding period on the following day after the injection of sugar plus extract 4.49 grams were recovered. Had we used the more powerful extract first, the reducing action might have been even more strikingly demonstrated.

We were surprised that we did not secure a raised blood sugar one hour after the first injection of sugar. The rise after the second injection was very marked. We gave the animal one day's rest and repeated the consecutive injections with the same results as above. The interpretation of the results of the first of the later injections is complicated by an unexplainably high percentage of sugar present before the injection. This phenomenon cannot be wholly explained by the rate of output of sugar since on the fourth injection, the first hour excretion was the maximum of the series and we did obtain a definite rise in blood sugar. An injection of 1 gram of sugar per kg. given to a normal dog showed a pronounced rise in percentage sugar of the blood after a 15-minute interval. This hyperglycemia rapidly subsided and at the end of an hour the blood sugar had regained its normal level; 2.29 grams of sugar were excreted.

Hemoglobin estimations were made (1) one hour after the first sugar injection, (2) just before the first injection of extract and (3) at 12 P.M., Oct. 9. The blood sugars at these times were .33 per cent, .35 per cent and .09 per cent respectively. The hemoglobin was identical at the second and third determinations; a slightly lower value was obtained at the first determination.

Chart 6 is the record of a short, but very interesting experiment which again demonstrates the remarkable effect of the extract of degenerated pan-

creas upon the power of a diabetic animal to retain sugar. On Nov. 8 at 11 A.M. (b.s. 35 per cent), 10 gm. of sugar were injected intravenously. The curve shows the rise in blood sugar. In the four hours following the injection, 10.88 gm. of sugar were excreted. From 3 to 9 P.M. 78 c.c. of dilute extract were injected in 13 c.c. doses. At 9 P.M. (b.s. .09 per cent), 10 gm. of sugar were injected. The curve shows the effect on blood sugar and sugar excretion. The effect of partially degenerated gland extract, 5 weeks after ligation of the ducts, upon the kidneys is shown here. This extract may produce a raised threshold to sugar or a condition of anuria, as in this experiment. Hemoglobin estimations before and after administration of extract were identical. Duodenal ulcer was the cause of the early termination of the experiment.

A more detailed description of the histologic sections obtained during our experiments will be included in a subsequent communication. Suffice it here to note that the pancreatic tissue removed after seven to ten weeks' degeneration shows an abundance of healthy islets, and a complete replacement of the acini with fibrous tissue.

In the course of our experiments we have administered over seventy-five doses of extract from degenerated pancreatic tissue to ten different diabetic animals. Since the extract has always produced a reduction of the percentage sugar of the blood and of the sugar excreted in the urine, we feel justified in stating that this extract contains the internal secretion of the pancreas. Some of our more recent experiments, which are not yet completed, give, in addition to still more conclusive evidence regarding the sugar retaining power of diabetic animals treated with extract, some interesting facts regarding the chemical nature of the active principle of the internal secretion. These results, together with a study of the respiratory exchange in diabetic animals before and after administration of extract, will be reported in a subsequent communication.

We have always observed a distinct improvement in the clinical condition of diabetic dogs after administration of extract of degenerated pancreas, but it is very obvious that the results of our experimental work, as reported in this paper do not at present justify the therapeutic administration of degenerated gland extracts to cases of diabetes mellitus in the clinic.

#### CONCLUSIONS

The results of the experimental work reported in this article may be summarized as follows:

Intravenous injections of extract from dog's pancreas, removed from seven to ten weeks after ligation of the ducts, invariably exercises a reducing influence upon the percentage sugar of the blood and the amount of sugar excreted in the urine.

Rectal injections are not effective.

The extent and duration of the reduction varies directly with the amount of extract injected.

Pancreatic juice destroys the active principle of the extract.

That the reducing action is not a dilution phenomenon is indicated by the



following facts (1) hemoglobin estimations before and after administration of extract are identical; (2) injections of large quantities of saline do not effect the blood sugar; (3) similar quantities of extracts of other tissues do not cause a reduction of blood sugar.

Extract made 0.1 per cent acid is effectual in lowering the blood sugar.

The presence of extract enables a diabetic animal to retain a much greater percentage of injected sugar than it would otherwise.

Extract prepared in neutral saline and kept in cold storage retains its potency for at least seven days.

Boiled extract has no effect on the reduction of blood sugar.

We wish to express our gratitude to Professor Macleod for helpful suggestions and laboratory facilities and to Professor V. E. Henderson for his interest and support.

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# THE EFFECTS OF VARIOUS FOODS, FOOD FACTORS AND CHEMICAL AGENTS UPON THE RESISTANCE OF ANIMALS TO ACETONITRILE\*

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(Received for publication, July 5, 1921)

THE correlation between the state of nutrition of animals and their resistance to poisons has claimed the attention of many investigators. La Monaco and Trambusti,<sup>1</sup> (1894) reported a difference in the toxicity of phosphorus in well nourished animals and in poorly fed ones. Foster<sup>2</sup> (1910) studied the resistance of dogs to ricin and artificial hemorrhage on various planes of protein intake but found no difference. Hunt<sup>3</sup> (1910) observed an increase in resistance in mice to acetonitrile when on a restricted diet. Ellinger<sup>4</sup> (1905) reported the failure of cantharides to produce injury to the kidneys of rabbits fed on carrots. Hunt noted striking alterations in the resistance of rats, mice and guinea pigs to poison, following a change in their diets. The bibliography on this general subject is extensive and varied and cannot be extended here.

Our ideas of what constitutes a food have changed decidedly in the past few years. One of the most notable advances relates to the importance of the quality of protein furnished in the food. Not only must proteins constitute a part of every diet, but they must be of a suitable amino acid make-up. In other words, the indispensability of certain amino acids in the diet has been emphasized. Again, the need of the mineral elements in the diet has been studied with the result that the relative importance of several of them is now better recognized. The knowledge of the vitamins, recently discovered and so widely studied at the present time, has revolutionized experimental feeding. Since an inadequate diet manifests its effects in altered metabolism, the influence of controlled intake, both qualitatively and quantitatively, in investigations upon the action of poisons in the body is of prime importance. It seemed desirable, therefore, to repeat some of the experiments on the effects of poisons in the light of the newer advances in the science of nutrition.

Hunt has shown that acetonitrile is a poison in the animal body and has stated that it acts as a very delicate test for certain changes in metabolism. The plan of the present experiments was to use as a basal diet a food mixture qualitatively and calorifically adequate, and to study the variation in resistance of mice to acetonitrile when the proportion of various constituents of the diet was varied as well as when other substances were added to the diet.

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## EXPERIMENTAL PART

The basal diet was made up as follows: Casein 31.0, butter fat 21.6, brew-ery yeast 2.0, salt mixture\* 6.8, and cornstarch 38.6 per cent.

The protein and starch had been extracted with alcohol to free them from accessory food substances. That this diet was adequate for maintenance or slight gain was shown by the fact that we have kept adult animals on it for seven months in good condition. In the tests of toxicity Kahlbaum's aceto-nitrile in distilled water was injected subcutaneously. Controls were used in each series.

As numerous experiments were performed, only the maximum nonfatal and the minimum fatal doses are given from one selected series of these repeated experiments in the following tables:

TABLE I

	BASAL DIET	DEFICIENCY IN WATER SOLUBLE VITAMIN B	DEFICIENCY IN FAT SOLUBLE VITAMIN A	DEFICIENCY IN BOTH VITAMINS	UNDERFEEDING
Change in Body Weight, gm.	17.8-20.2 (+12%)	18.3-14.1 (-24%)	17.9-17.0 (-5%)	18.5-14.4 (-23%)	18.3-16.6 (-10%)
Duration of Feeding, days	22-28	22-28	22-28	22-28	22-28
Number of Mice Tested	9	9	9	9	9
Acetonitrile (mg. per gm. mouse)					
Max. Nonfatal	0.23	0.28	0.22	0.28	0.26
Min. Fatal	0.23	0.20	0.23	0.25	0.25

## EFFECT OF LACK OF VITAMIN

Mice fed upon the above mixture without yeast, i.e., on a diet deficient in vitamin B, lost weight after an initial maintenance or slight gain. Though the effect of deficiency in vitamin A, secured by replacing butter fat in the basal diet with lard, was slower in making itself evident, our results plainly indicate the indispensability of both vitamins to adult as well as to growing mice. Acetonitrile was injected at varying intervals in different mice after the decline in weight appeared. As seen from Table I deficiency or lack of one or both vitamins had no effect on the resistance of mice to poisoning with acetonitrile.

## EFFECT OF UNDERFEEDING

It is often stated that temporary starvation has a beneficial effect in many diseases. Experiments with underfeeding were carried out in connection with acetonitrile tests similar to those of Hunt but with more accurately controlled food intake. When the experimental mice were fed one-fourth of the food eaten by the control mice they lost weight quite rapidly. To avoid such unduly severe loss in weight the mice were therefore given one-half the amount of food eaten by the control animals. Except in the case where rolled oats

\*Osborne and Mendel: Jour. Biol. Chem., 1918, xxxiv, 131. The lactose was omitted.

and water alone were given, underfeeding did not decrease the toxicity of acetonitrile, unless the animals were fed anew small quantities of the food just a few hours before the injection. (See Table II.)

TABLE II

	LOW PROTEIN DIET*		SALT-POOR DIET		OATS DIET	
	CONTROL	UNDER- FEEDING	CONTROL	UNDER- FEEDING	CONTROL	UNDER- FEEDING
(A) ACETONITRILE INJECTION WITHOUT PREVIOUS FEEDING.						
Change in Body Weight, gm.	21.7-15.4 (-30%)	20.6-14.0 (-32%)	21.3-21.1 (-1%)	22.4-18.0 (-20%)	16.2-15.2 (-6%)	15.1-12.1 (-20%)
Duration of Feeding, days	11	11	11	11	11	11
Number of Mice	6	6	5	5	5	5
Acetonitrile						
Max. Nonfatal	0.32	0.32	0.38	0.28	0.40	0.64
Min. Fatal	0.30	0.30	0.40	0.32	0.44	0.70
(B) ACETONITRILE INJECTION AFTER PREVIOUS FEEDING.						
Change in Body Weight, gm.	23.1-23.5 (+1%)	23.2-19.8 (-14%)	14.1-14.4 (+2%)	14.3-12.4 (-13%)		
Duration of Feeding, days	10	10	10	10		
Number of Mice	6	7	6	7		
Acetonitrile						
Max. Nonfatal	0.35	0.45	0.12	0.25		
Min. Fatal	0.40	0.50	0.14	0.24		

\* The composition of the diet is shown in Table III, footnote.

## EFFECT OF RATE OF GROWTH

Hunt stated that in his experiments the greater the rate of growth the greater was the susceptibility of the animal to acetonitrile; whereas animals increasing in weight slowly, showed relatively greater resistance to it. However, in our experiments with two groups of young mice, one group growing faster than the other, no difference in the resistance to acetonitrile was observed.

## EFFECT OF DIETS LOW IN PROTEIN

Salant and Rieger<sup>5</sup> (1917) reported that with diets low in protein, an increased resistance to the effects of caffeine was observed. In one of our series of experiments we substituted carbohydrate isodynamically for protein, and fat for protein in another series. Hunt reported increased resistance when various carbohydrates were superimposed upon his cake diet.

TABLE III

	BASAL DIET	LOW PROTEIN (HIGH CARBOHYDRATE)*
Change in Body Weight, gm.	17.5-19.0(+8%)	17.9-18.2(+1%)
Duration of Feeding, days	14	20
Number of Mice	21	9
Acetonitrile		
Max. Nonfatal	0.40	0.48
Min. Fatal	0.40	0.50

\*Casein 6.0, dextrin 25.0, butter fat 21.6, brewery yeast 2.0, salt mixture 6.8 and cornstarch 38.6 per cent.

TABLE IV

	BASAL DIET	LOW PROTEIN (HIGH FAT)**
Change in Body Weight, gm.	16.4-17.1 (+3%)	16.4-13.1 (-20%)
Duration of Feeding, days	12	12
Number of Mice	6	6
Acetonitrile		
Max. Nonfatal	0.56	0.40
Min. Fatal	0.60	0.40

\*\*Casein 4.0, lard 13.5, butter fat 21.6, brewery yeast 2.0, salt mixture 6.8 and cornstarch 38.6 per cent.

As will be seen from Tables III and IV the low protein diet in which carbohydrate is substituted for some of the protein increased the resistance of the mice somewhat while the diet in which protein was largely replaced by fats decreased the resistance of the mice. A distinct tendency to lose weight was noticed in the fat experiments.

## EFFECT OF DIETS HIGH IN FATS

Salant and Nelson (1917) found a modification of the toxicity of chenopodium oil in cats by feeding coconut and cottonseed oils. Hunt reported that a high fat diet rendered mice less resistant to acetonitrile. In the diet used in the present experiment a large part of the carbohydrate was replaced isodynamically with lard. As seen from Table V our results show that on a diet high in fat mice are slightly less resistant to acetonitrile. We have found this true especially after a long period of eating such a diet.

TABLE V

	BASAL DIET	FAT DIET*
Change in Body Weight, gm.	25.5-27.5 (+8%)	24.9-27.3 (+9%)
Duration of Feeding, days	33	33
Number of Mice	5	5
Acetonitrile		
Max. Nonfatal	0.32	0.22
Min. Fatal	0.34	0.24

\*Casein 25.0, butter fat 21.6, lard 18.0, brewery yeast 2.0, salt mixture 6.8 and cornstarch 8.6 per cent.

## EFFECT OF KIND OF FAT

Since the character of the fat deposited in the body is dependent, to some extent, upon the nature of the fat fed, and since various fats may conceivably be oxidized with different facility, it seemed of interest to try the effect of fats of various kinds to see whether or not the ease of fat oxidation in the

TABLE VI

	BASAL DIET	COD LIVER OIL DIET*	OLIVE OIL DIET*
Change in Body Weight, gm.	17.9-19.8 (+10%)	18.0-18.2 (+1%)	17.8-17.8 ( $\pm 0\%$ )
Duration of Feeding, days	14	14	14
Number of Mice	7	8	8
Acetonitrile			
Max. Nonfatal	0.60	0.40	0.40
Min. Fatal	0.55	0.45	0.45

\*Two-thirds the amount of butter fat in the basal diet were replaced with oils under investigation.



body had any influence upon the simultaneous oxidation of acetonitrile. Butter fat, olive oil and cod-liver oil were the fats used. From Table VI it is seen that olive oil and cod-liver oil tend to lower the resistance slightly.

#### EFFECT OF VARIOUS ANIMAL TISSUES

Since many of the organs are more or less closely associated with growth, nutrition and immunity, the effect of feeding various organs on the resistance to acetonitrile was tested. Hunt has reported the effects with various tissues but the result with thyroid was most striking. In the present experiments tissues were fed in small quantities varying from 0.1 to 3.0 gms. of dried tissue-substance in 100.0 gms. of basal diet and replacing an amount of protein calculated from the factor  $N \times 6.25$ . The tissues were kidney, pancreas, sub-maxillary, parotid, ovary, lymph gland, suprarenal, brain, heart muscle, skeletal muscle, and pituitary. None of these had any effect on the resistance to acetonitrile except kidney which seemed to lower it. When preparations from fresh lamb kidney and beef liver were fed, no effect on the resistance was observed. In our trials with tissues added to the diet, there have been no instances of increased resistance to acetonitrile except in the case of the thyroid which will be discussed elsewhere.

#### EFFECT OF MINERAL ELEMENTS

The literature on salt metabolism and mineral requirements in nutrition is too extensive to review here. The problem of salt action in the body, the rôle of salts in absorption and osmotic phenomena, the part played by salt ions in conduction and irritability and the importance of salts in the acid-base equilibrium in the blood add importance to the physiology of mineral elements in the body. In the present experiments, diets poor in Ca, Cl,  $\text{SO}_4$ ,  $\text{PO}_4$ , K, Mg, and Na, respectively, were used. These various salt mixtures were prepared, exactly following the plan of Osborne and Mendel (1918) for studies of inorganic elements in nutrition. In addition, diets poor in salts,\* free from iodine and with iodine alone were used. The results with food lacking one or other of the elements were so variable that they were insignificant. With the salt-poor and iodine-free diets, the resistance to acetonitrile was diminished while with the iodine diet loss of resistance was less. Hunt also found that iodine seemed to impart some variable degree of resistance to the poison.

#### EFFECT OF KIND OF PROTEIN

Since some of the various amino-acids are structurally similar to active components of some of the internal secretions, and since it seems that in its toxicity in the animal body acetonitrile involves processes governed by these internal secretions, at least in part, it seemed important to study the effect of variation in the composition of the proteins making up the diet. Gelatin, casein and edestin were tested. Tyrosine was used to supplement the gelatin and in some of the experiments, casein and gelatin were fed together. When gelatin and gelatin-tyrosine were the sole source of protein in the diet there

\*Salt mixture was omitted from the basal diet.

was an increase in resistance to acetonitrile. When edestin or gelatin-casein were the source of protein there was no increase in the resistance to the poison. Hunt reported experiments which ours seem to corroborate with protein in general.

#### EFFECT OF OAT STARCH

According to Hunt, mice fed upon oatmeal or oats exhibit an increase in resistance to acetonitrile. He attributed this beneficial effect to the increased ability of the thyroid to take up iodine after feeding with oats.

In the present experiments, oat starch was prepared from rolled oats. Mice fed with the oat starch diet showed a remarkable resistance to acetonitrile. When, however, the starch was first extracted with alcohol and ether, there was no increase in the resistance to the poison. The ash of the oats likewise had no effect in altering the susceptibility to the poison.

#### CONCLUSION

The foregoing experiments seemed warranted from the standpoint of the use of improved technic. We have employed standardized, synthetic foods, the nutritional significance of every factor of which is known. This has enabled us to vary the constituents of the food at will while keeping the composition of the food under exact control. The experimental evidence shows that only in the cases of underfeeding, of feeding with iodine as the sole mineral, of feeding with oat starch and possibly of feeding with diets high in fats, was any alteration in the resistance to acetonitrile secured.

It appears therefore that the susceptibility to poisoning in mice with acetonitrile is not easily affected by changes in dietary composition within wide limits of quantity and especially of quality. Perhaps this may serve to emphasize the factor of safety possessed by the organism in its response to alterations of the diet.

The author desires to express his thanks to Professor Lafayette B. Mendel for his helpful advice and criticism.

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## EFFECT OF TIME BETWEEN OBTAINING A SPINAL FLUID AND MAKING A CELL COUNT ON THE RESULT OF THE COUNT\*

BY JAMES WYNN, M.D., BOSTON, MASS.

MOST writers in discussing the Fuchs-Rosenthal counting chamber and cerebrospinal fluid cell counting lead one to the inference that cells must be counted immediately after lumbar puncture if the count is to be relied upon. Boyd,<sup>1</sup> in referring to the Fuchs-Rosenthal method, mentions as a great practical advantage that it is usable at the bedside. Then follows the statement: "Indeed, the sooner the count is made after the withdrawal of fluid the better, as sedimentation occurs rapidly and the cells are liable to adhere to the sides of the tube." Kolmer<sup>2</sup> and others<sup>3,4</sup> have also specified that the fluids for counting must be fresh.

This is obviously true enough in most cases in which there is a very high degree of meningeal irritation. It is a matter of common experience for such fluids to show webs even before they can be rushed to the laboratory. Evidence, however, seems lacking to prove immediate counting necessary in case of normal spinal fluids or those with more moderate pleocytoses such as are characteristic of a large group of tabetics and neurosyphilities.

In view of this scarcity of data, the cells in eighty spinal fluids from the wards of this hospital were counted at varying intervals (see Table I) up to fifteen hours after lumbar puncture. The fluids were collected (6 c.c. to the tube) in clean (not sterile) test tubes (10 × 100 mm.) and these were tightly stoppered with corks. Before each count the tubes were gently inverted twenty to thirty times. A Levy double counting chamber was used and the cells in eighteen square millimeters of fluid counted in each case. (Slight variations in the treatment of the fluid immediately before counting are explained in the legend of Table I.) During the intervals between counts, the first forty fluids of the series were placed in the ice box; the remainder were allowed to remain at room temperature. The counts are tabulated in Table I.

The constant association of macroscopic changes in the fluid with subsequent variations from the bedside cell count would seem to suggest that purely mechanical factors are responsible for such changes in count, and at Dr. Christian's suggestion it was decided to mix in a small quantity of powdered sodium citrate with one of the specimens collected in every case presenting on puncture any gross abnormality in the fluid, in order to see whether clotting might not be an important factor in causing the change in the cases and to see whether preventing the clotting might not prevent the occurrence of the change. Unfortunately, there have been only two such

\*From the Medical Service of the Peter Bent Brigham Hospital, Boston, Mass.

TABLE I

NAME	DIAGNOSIS	SPINAL FL. WASSERMANN	GLOB- ULIN	IMMEDI- ATELY	MACROSCOPIC APPEARANCE ON STANDING	CELL COUNT 0 HR	1 HR	2 HR	3 HR	6 HR	10 HR	12 HR	15 HR
L.A.S.	syphilis of c. n. s.	0.2 c.c. ++	++	clear	clear	A-3 P-3		A-3 P-4				A-3 P-3	
L.F.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	A-17 P-18		A-16 P-17				A-15 P-19	
R.P.	encephalitis lethargica	2.0 c.c. ±	-	clear	clear	A-270 P-276			A-280 P-178			A-281 P-280	
W.G.	encephalitis lethargica	1.0 c.c. ±	+	clear	clear	A-89 P-89			A-87				A-89
M.Z.	syphilitic meningi- tis (treated)	1.5 c.c. -		clear	clear								
		0.2 c.c. ++	++	clear	clear	O 10 5-12		O 9 5-12					O-11 5-13 spl1
M.S.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	sp10 O-33		sp11 O-35				O-34 5-31	
R.B.	tabes dorsalis	0.3 c.c. ++	++	clear	clear	5-29 5-126		5-21	5-128				5-124
K.L.	acute anterior poliomyelitis	2.0 c.c. -	±	clear	hazy in 5 hr.	P-126 P-148				P-132 A-124			
R.E.L.	syphilis of c. n. s.	0.6 c.c. ++	++	clear	clear	A-146 P-19			P-18 A-17			P-17 A-17	
R.C.	tabes dorsalis	0.7 c.c. ++	++	clear	clear	A-18 P-34		A-31			A-32		
J.L.	encephalitis lethargica	2.0 c.c. -	++	clear	sed. in 5 hr.	A-34 P-170				P-150 A-148			P-10 A-10
O.L.	syphilis of c. n. s.	0.6 c.c. ++	++	clear	clear	A-170 P-10			P-9 A-10				
A.R.	syphilis of c. n. s.	0.3 c.c. ++	++	clear	clear	A-10 P-7		P-7 A-6				P-6 A-5	
O.C.	syphilis of c. n. s.	0.6 c.c. ++	++	clear	clear	A-7 P-11			P-12 A-11				P-11 A-10
J.P.	gastric neurosis	2.0 c.c. -	-	clear	clear	A-11 P-1			P-2 A-2				P-1 A-1
J.O.	diabetes mellitus	2.0 c.c. -	-	clear	clear	A-0 A-1			P-2 A-1			P-1 A-0	



TABLE I—CONTINUED

NAME	DIAGNOSIS	SPINAL FL. WASSERMANN	GLOB- ULIN	IMMEDI- ATELY	MACROSCOPIC APPEARANCE	CELL COUNT 0 HR	1 HR	2 HR	3 HR	6 HR	10 HR	12 HR	15 HR
H.E.L.	syphilis (not of c. n. s.)	2.0 c.c. —	—	clear	clear	P-3 A-3			P-2 A-2				P-3 A-2 A-58
R.P.	encephalitis lethargica	2.0 c.c. ±±	—	clear	pellicle in 5 hr.	P-53 A-50				P-90 A-63			
R.S.	neurasthenia	2.0 c.c. —	—	clear	clear	P-6 A-4			P-5 A-4				P-4 A-3 A-3
F.B.	mitral stenosis	2.0 c.c. —	—	clear	clear	P-3 A-3				P-3 A-4			
R.P.	encephalitis lethargica	2.0 c.c. —	±±	clear	pellicle in 1 hr.	A-53 P-54		A-62 P-90				A-58 P-65 A-1 P-1	
M.C.	syphilis (not of c. n. s.)	2.0 c.c. —	—	clear	clear	A-2 P-2				A-1 P-2			
L.H.	syphilis of c. n. s.	0.5 c.c. ±±±	++	clear	clear	A-7 P-9		A-8 P-8				A-7 P-8	
G.S.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	A-5 P-7				A-6 P-7 A-12 P-14		A-6 P-6	
G.Su.	tabes dorsalis	0.7 c.c. ++	++	clear	clear	A-11 P-13							A-12 P-16
E.L.	syphilis of c. n. s.	0.8 c.c. ++	±±	clear	clear	A-15 P-18					A-19 P-21		
J.N.	syphilis of c. n. s.	0.8 c.c. ++	++	clear	clear	A-14 P-15		A-14 P-13					
O.C.	syphilis of c. n. s.	0.4 c.c. ++	++	clear	clear	A-10 P-10						A-12 P-12 A-8 P-10 A-6 P-7 A-7 P-10	A-8 P-8 A-11 P-12
M.Z.	syphilitic menin- gitis (treated)	0.4 c.c. ++	++	clear	clear	A-7 P-9	A-7 P-8						
R.B.	tabes dorsalis	0.4 c.c. ++	++	clear	clear	A-8 P-10			A-7 P-9 A-7 P-9				
G.S.	tabes dorsalis	0.6 c.c. ++	++	clear	clear	A-9 P-9							
J.W.	c. n. s. lues	0.4 c.c. ++	++	clear	cloudy in 3 hr.	A-30 P-32			A-16 P-19	A-16 P-18		A-11 P-12	
O.C.	syphilis of c. n. s.	0.4 c.c. ++	++	clear	clear	A-12 P-15							

TABLE I—CONTINUED

NAME	DIAGNOSIS	SPINAL FL. WASSERMANN	GLOB- ULIN	MACROSCOPIC APPEARANCE IMMEDI- ATELY	STANDING	CELL COUNT 0 HR.	1 HR.	2 HR.	3 HR.	6 HR.	10 HR.	12 HR.	15 HR.
L.S.	neurosis	2.0 c.e. -	-	clear	clear	A-2 P-2			A-0 P-1			A-1 P-1	
L.H.	syphilis of c. n. s.	0.6 c.e. ++	++	clear	clear	A-10 P-12		A-11 P-12				A-11 P-13	
A.G.	psychoneurosis	2.0 c.e. -	-	clear	clear	A-5 P-5	A-4 P-5					A-4 P-4	
E.H.	luetie myelitis (treated)	0.6 c.e. ++	++	clear	clear	P-19 A-17						A-17 P-17	
G.S.	tabes dorsalis	0.6 c.e. ++	++	clear	clear	A-15 P-16						A-14 P-14	
X.R.	acute anterior poliomyelitis	2.0 c.e. -	++	clear	sed. = 12 hr.	O-230 5-232 sp230	O-228 5-230 sp234		O-230 5-238 sp226			O-180 5-174 sp176	
S.L.	acute anterior poliomyelitis	2.0 c.e. -	+-	clear	central web = 3½ hr.	O-17 5-17 sp15	O-18 5-17 sp16	O-16 5-14 sp17					
R.B.	neurosis	2.0 c.e. -	-	clear	clear	P-1 A-1					P-3 A-1		
M.Z.	syphilitic menin- gitis (treated)	0.4 c.e. ++	++	clear	clear	P-16 5-18 sp16			P-20 5-20 sp19			P-16 5-17 sp16	
R.B.	tabes dorsalis	0.4 c.e. ++	++	clear	clear	P-63 5-57 sp55			P-61 5-64 sp62				P-58 5-61 sp57
O.C.	syphilis of c. n. s.	0.4 c.e. ++	++	clear	clear	P-22 A-20				P-19 A-19			P-21 A-20
J.W.	syphilis of c. n. s.	0.4 c.e. ++	++	clear	clear	P-4 A-4		P-4 A-3					P-4 A-4
H.S.S.	tabes dorsalis	0.6 c.e. ++	++	clear	clear	P-17 A-16			P-15 A-16 P-27			P-17 A-15	P-27 A-27
J.P.O.	tabes dorsalis	1.0 c.e. ++	++	clear	clear	P-25 A-23							P-2 A-1
L.S.	syphilis (not of c. n. s.)	2.0 c.e. -	-	clear	clear	P-2 A-1							

TABLE I—CONTINUED

NAME	DIAGNOSIS	SPINAL FL. WASSERMANN	GLOB- ULIN	MACROSCOPIC APPEARANCE	CELL COUNT 0 HR	1 HR	2 HR	3 HR	6 HR	10 HR	12 HR	15 HR
F.D.	neurosis	2.0 c.e. -	-	clear	P-3 A-3		P-2 A-3				P-3 A-2 P-85 A-84	
J.Q.	syphilis of c. n. s. (paresis)	0.2 c.e. ++	++	clear	P-78				P-77 A-75			
C.B.	encephalitis lethargica	2.0 c.e. -	-	clear	A-76 A-30 P-30			A-27 P-31				A-27 P-25
R.B.	tabes dorsalis	0.3 c.e. ++	++	clear	P-60 A-60					P-61 A-58		
K.L.	acute anterior poliomyelitis	0.2 c.e. -	++	clear	P-140 5-130 sp130	P-144 5-135 sp130	P-118 5-110 sp112	P-120 5-114 sp115			P-41 5-36 sp38	
G.N.	syphilis of c. n. s.	0.5 c.e. ++	++	clear	P-10 5-11 sp11	P-10 5-11 sp10	P-14 5-15 sp12	P-15 5-10 sp11				P-10 5-12 sp10 P-6 5-4 sp5
E.Q.	lateral sclerosis	1.0 c.e. ++	++	clear	P-3 5-4 sp3	P-5 5-5 sp4	P-5 5-4 sp4					
C.C.	acute anter. poliomyelitis	2.0 c.e. -	+ -	clear	P-22 5-23 sp21	P-22 5-23 sp21	P-22 5-23 sp21	P-18 5-22 sp23		P-21 5-21 sp17		
M.M.	acute anterior poliomyelitis	2.0 c.e. -	++	clear	P-232 5-236 sp238	P-238 5-238 sp242	P-240 5-236 sp240				P-80 5-78 sp76	
M.Z.	encephalitis lethargica	2.0 c.e. -	+ -	clear	P-2 A-2			P-2 A-1				P-2 A-2 P-26 A-25
F.V.	encephalitis lethargica	0.5 c.e. ++	-	clear	P-27 A-26		P-26 A-25					
G.L.	tabes dorsalis	0.5 c.e. ++	++	clear	A-6 P-6			A-6 P-8				
G.S.	tabes dorsalis	0.6 c.e. ++	++	clear	A-16 P-15				A-15			
L.S.	tabes dorsalis	0.6 c.e. ++	++	clear	P-14 A-14			A-16				A-14
A.F.	aortic insufficiency	2.0 c.e. -	-	clear	P-2 A-2					A-2		A-3

TABLE I—CONTINUED

NAME	DIAGNOSIS	SPINAL FL. WASSEMANN	GLOB- ULIN	MACROSCOPIC APPEARANCE IMMEDI- ATELY	ON STANDING	CELL COUNT 0 HR	1 HR	2 HR	3 HR	6 HR	10 HR	12 HR	15 HR
S.S.	syphilis of c. n. s.	0.4 c.e. ++	++	clear	clear	P-32 A-30				A-32			A-32
H.D.	syphilis of c. n. s.	0.6 c.e. ++	++	clear	clear	P-7 A-7			A-7				A-6
H.W.	syphilis of c. n. s.	0.4 c.e. ++	++	clear	clear	P-11 A-10				A-9			A-9
E.R.	tabes dorsalis	0.5 c.e. ++	++	clear	clear	P-15 A-14				P-14 A-14			A-13
P.R.	syphilis (not of c. n. s.)	2.0 c.e. —	—	clear	clear	P-1			A-1				A-1
C.T.	syphilis (not of c. n. s.)	2.0 c.e. —	—	clear	clear	A-1				P-1			A-1
A.F.	syphilis of c. n. s.	0.5 c.e. ++	++	clear	clear	P-50 A-48		P-49 A-48				P-49 A-50	
H.W.	syphilis of c. n. s.	0.5 c.e. ++	++	clear	clear	A-6			A-5 P-6				A-6 P-6
J.N.	syphilis of c. n. s.	0.8 c.e. ++	++	clear	clear	A-69 P-69				A-69 P-68			A-68 P-68
W.S.	tabes dorsalis	0.8 c.e. ++	++	clear	clear	A-5 P-5			A-4 P-4				A-5 P-4
B.B.	chronic diarrhea	2.0 c.e. —	—	clear	clear	P-1				P-0 A-0			P-1 A-0
H.D.	syphilis of c. n. s.	0.6 c.e. ++	++	clear	clear	A-1 P-5			P-4 A-4				A-3 P-4
W.S.	tabes dorsalis	0.8 c.e. ++	++	clear	clear	A-4 P-10				P-10 A-9			P-9 A-9
C.N.	syphilis of c. n. s.	0.4 c.e. ++	++	clear	clear	A-8 P-25		P-25 A-24				P-24 A-24	
A.D.	syphilis of c. n. s.	0.5 c.e. ++	++	clear	clear	A-23 P-5				P-4 A-3		P-5 A-5	
J.N.	syphilis of c. n. s.	0.8 c.e. ++	++	clear	clear	P-18 A-16					P-16 A-14		P-17 A-17
W.S.	tabes dorsalis	0.8 c.e. ++	++	clear	clear	P-13 A-11				P-11 A-10			P-12 A-11



cases subsequently on the wards over a period of many months. The results of counting after such a procedure in these two cases are shown in Table II.

TABLE II

GROSS APPEARANCE—TYPE IV PNEUMOCOCCUS MENINGITIS			
Tube plain fluid immediately opalescent	on standing central funnel web in one hour	Tube citrated fluid immediately opalescent	on standing no change in 15 hours
CELL COUNTS			
	at 0 hr	at 3 hrs.	at 15 hrs.
plain tube	3000	impossible in one hr. because of web	not possible
citrated tube	3000	2990	2994
GROSS APPEARANCE—SYPHILITIC MENINGITIS			
Tube plain fluid immediately opalescent	on standing unchanged in 15 hr.	Tube citrated fluid immediately opalescent	on standing no change in 15 hr.
CELL COUNTS			
	at 0 hr	at 3 hrs.	at 15 hrs.
plain tube	2500	2510	2489
citrated tube	2504	2490	2496

Note:—All counts made with undiluted fluids.

The following conclusions seem warranted from the foregoing data:

(1) The cells in clear spinal fluids, collected in clean tubes and tightly stoppered, may in the absence of macroscopic pellicle, sediment, or web be safely counted if thoroughly mixed at any time up to at least fifteen hours. If the spinal fluid is not clear or becomes clouded, the results of counts at different times are apt to vary.

(2) It is desirable but not necessary for fluids to be collected in sterile tubes.

(3) Whether the fluids are kept at room temperature or in the ice box seems to be of little consequence.

(4) In two cases of meningitis, the thorough mixture of small quantities (0.2-0.5 gm. for 6 c.c.) of powdered sodium citrate with the fluids made it possible to duplicate the original cell counts (within the limit of technical error) at three and fifteen hour intervals. The procedure must be tried in a large series of cases before any conclusions are warranted regarding such citrating of fluids.

## REFERENCES

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- <sup>3</sup>Emerson, C. P.: Clinical Diagnosis, ed. 4, p. 700.
- <sup>4</sup>With, C.: Brain, 1917, xl, 403.
- <sup>5</sup>Webster, R. W.: Diagnostic Methods, ed. 5, p. 682.

# ON THE DETERMINATION OF SMALL QUANTITIES OF ATROPINE IN BLOOD SERUM\*

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IN PREPARING a paper on the natural immunity of the rabbit for atropine which has appeared in an abbreviated form in the *Arch. Néerl. de Physiologie* (T V, 3 me Livr., p. 380, 1921), I had to examine all alkaloid reagents with regard to their applicability for the quantitative determination of atropine, because I found that no one had done this before me. As I think that this preliminary work, which cost me much time, may be of some use to later students of this subject, I publish herewith its results.

The determination of small quantities of the alkaloid in blood serum can immediately be divided into two parts: (1) The isolation of the alkaloid out of the complicated chemical mixture which we call serum. (2) The determination of atropine in the watery solution thus obtained. I shall treat these two parts separately.

## 1. ISOLATION OF THE ALKALOID

In forensic chemistry such a case would demand the method of Stass-Otto, which has been accurately described in the *Lehrbuch der gerichtlichen*

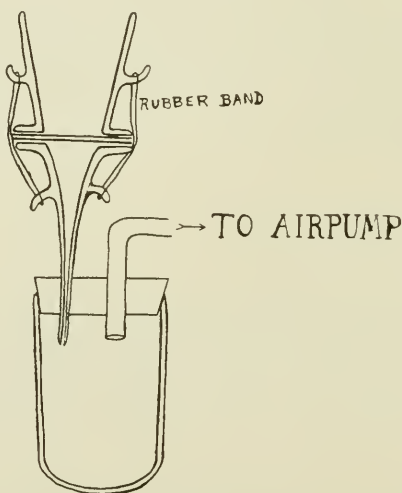


Fig. 1.—Apparatus for microfiltration. The liquid passes through two pieces of filter paper kept tightly between the two pieces of the apparatus by means of rubber bands.

*Medizin* of Baumert. Without comparing it in detail with the other existing methods, as for instance that of Kippenberger—the bibliography showed me that one of them is as good and bad as the other—I immediately tried to

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adapt this method to microchemical analysis. Using small test tubes, containing about 15 c.c., with a thick wall, so that they could be used in the centrifuge, the amount of atropine was determined in one c.c. of serum, containing various amounts of atropine dissolved in one c.c. of water. Immediately after they had been taken out of the incubator, they were filled with absolute alcohol, and thoroughly shaken. The precipitate was collected on the bottom by means of a strong centrifuge, and the completely clear supernatant liquid decanted. Then the proteins were washed out several times with absolute alcohol, and all the alcohol united in a beaker. As I usually had a number of determinations to do at the same time, I boiled them all down on a large sand-bath. In this case one must be very careful to prevent bumping and to take them off at the very moment that they get dry, otherwise the material is roasted, and the determination spoiled.

The residue is now taken up in water and boiled down to a few c.c. Droplets of fats appear as soon as the preparation is allowed to cool. The liquid, in which a small precipitate sometimes has been formed, principally consisting of fat, is now filtered off by means of a microfilter (Fig. 1). The volume of the watery atropine-solution now obtained—which is completely clear and contains moreover perhaps some inorganic salts and some amino-acids in negligible quantities—was determined by weighing.\*

## 2. DETERMINATION OF THE QUANTITY OF ATROPINE IN THE SOLUTION

The methods of determination of atropine can be divided into five groups. They are: A. Physiological methods. B. Color reactions. C. Precipitate reactions. D. Titrimetrical methods. E. Capillary-analytical methods.

A. Of these I know five which could be used for our purpose. A very sensitive method has been devised by Fleischmann,<sup>1</sup> by which even  $10^{-8}$  gm. can be detected. In the frog heart, which has been inhibited by means of muscarine, he injected by means of a Pravaz-syringe the mixture of serum and atropine, in which the quantity had to be determined. This method can, however, not be used for our purpose because the quantities of atropine and muscarine, which compensate each other, are different in different cases. However accurate this method may be it can only be used to detect very small quantities qualitatively. The second method is that of Metzner,<sup>2</sup> who used the stimulation of the N. vagus for this purpose. This method is as well as that of measuring of the mydriasis, the third one, not sensitive enough for our purpose. In the fourth place I wish to call the attention to the work of Storm van Leeuwen,<sup>3</sup> who working in this line used the movements of a surviving gut for the estimation of the alkaloid, according to the method of Magnus. This method as well as that of Cushny<sup>4</sup> who uses a dog with a salivary fistula, can only be used for a few experiments; when a whole series of estimations has to be made, both take too much time and material. Moreover these methods have always the disadvantage of depending on many other factors (tem-

\*In order to be sure, that in this way all the atropine was obtained, I made a control experiment in the following way. The precipitates of about ten determinations were collected, boiled and extracted with about all atropine-solvent reagents, such as alcohol, ether, water and hydrochloric-alcohol. The whole mass was then neutralized, boiled down in the same way as above described, and taken up in a few drops of water. No atropine could be demonstrated herein.

perature, condition of the animal, etc.), so that for an accurate quantitative determination we can better use a chemical method.

B. There are a great number of qualitative color reactions for alkaloids. For quantitative purposes with reference to atropine, none of them can be used as the following review will show.

1. *The reaction of Vitali*.—This is the most well known of the color reactions for atropine.<sup>5</sup> The solution is evaporated on a water-bath with concentrated nitric acid. After it has been allowed to cool, alcoholic potassium hydroxide is added. The solution gets a red color, if atropine is present. There is however no possibility to adapt this method to quantitative purposes—for instance with a color scale or with a colorimeter, because as I could state several times, the color changes every moment. In accepting the results which several authors obtained in using this reaction, one should for this reason be very careful.

2. *The reagents of Wasicky*.<sup>6</sup>—A. With perhydrol-sulphuric-acid the three alkaloids, atropine, hyoseyamine and scopolamine assume a color, which is first leaf-green, then olive-green and finally brown-green. For the same reason as mentioned for the reaction of Vitali, this method cannot be adapted for quantitative use. That atropine should assume a color, when treated with perhydrol-sulphuric-acid, is, however, denied by Springer (Pharm. Ztg., 1902, p. 157), neither could I see any coloration. Probably the color seen by Wasicky is due to impurities. B. The reaction with p-dimethylbenzaldehyde—a cherry-red color—is still more sensitive. In how far this reaction can be adapted for quantitative use, must still be investigated. The author ascertains that the color remains constant for days. As it is practically impossible to obtain this reagent in sufficient quantities, we may safely exclude it for laboratory-practice.

3. *Sulphuric acid*, mentioned by several authors (recently Tunmann<sup>7</sup>) as a color reagent to alkaloids in general and to atropine particularly, gives, as already stated by Eder,<sup>8</sup> no color with atropine, not even when it is mixed with  $H_2O_2$  (*perhydrol-sulphuric-acid*), with ammonium- or alkali-persulphate (*the reagent of Caro*), with  $KMnO_4$  (1 gm. in 200 c.c.  $H_2SO_4$ ) (*the reagent of Wenzel*), with  $HNO_3$  (*the reagent of Erdman*), with a trace of iron (*the reagent of Keller*), with a watery solution of permanganate (*the reagent of Flückinger*), or with benzaldehyde (in alcoholic solution 1:5) (*the reagent of Melzer*).

4. *Nitric acid*, mentioned several times as a general alkaloid-reagent, gives no coloration with atropine.

5. *The reagent of Schlagdenhauffen*, a pyrogallol-solution, gives no color with atropine, neither when the solution is warmed.

6. *The reagent of Selmi*, a saturated solution of iodic acid, gives with some alkaloids a color reaction, due to the reduction of iodic acid.\* Atropine, however, seemingly does not give this reaction.

7. *The reagent of Watson-Smith*,  $SbCl_3$ , colors many alkaloids. Atropine is not mentioned among them. Moreover this reagent is rather dangerous, as  $SbH_3$  is easily formed.

\*Stein<sup>9</sup> worked out a colorimetric method based upon this reaction.



8. *The reagent of Fraude*, perchloric acid with a specific weight of 1.13-1.14 cannot be used for atropine. The author used it especially for aspidospermine and the Strychnos-alkaloids. According to Häussermann and Sigel (Chem. Ztg., 1901, p. 32) pure perchloric acid (prepared from Ag-perchlorate and  $\text{H}_2\text{SO}_4$ ) does not give this reaction. It is probably due to traces of Cl or  $\text{ClO}_2$ .

9. *The reagent of Lenz*, KOH, gives no color with atropine.

10. *The reagent of Archetti* (Ztschr. f. anal. Chem., 1901), a solution of potassium ferrieyanide in  $\text{HNO}_3$ , gives an olive-brown color with atropine. This reagent has been used for quantitative purposes by Kieffer, but seems not to be very reliable.

11. *The reagent of Lucchini* (Chem. Kal., II, 695), a solution of potassium bichromate in concentrated  $\text{H}_2\text{SO}_4$  gives no color with atropine.

12. The following reagents have been mentioned in the literature as untrustworthy, some of them have been excluded for practical reasons: *Urbansky and Mylius* (5 drops of furfural in 10 c.c.  $\text{H}_2\text{SO}_4$ ), *Frödhe or Buckingham* (1 gr.  $(\text{NH}_4)_2\text{MoO}_4$  in 100 c.c.  $\text{H}_2\text{SO}_4$ ), *Mandelin* (1 gm. ammonium-vanadium-sulphate in 200 c.c.  $\text{H}_2\text{SO}_4$ ), *Sonnenschein* (ceriums sesquioxide in  $\text{H}_2\text{SO}_4$ ), *Lafon or Ferreira de Silva* (ammonium selenite in conc.  $\text{H}_2\text{SO}_4$ ), and the reaction of *Schneider*, using  $\text{H}_2\text{SO}_4$  and sugar, the color being due to the formation of furfural.

13. CN-gas colors a concentrated alcoholic atropine solution reddish-brown.<sup>10</sup> As our purpose is to determine small quantities, we can exclude this reaction.

Considering our results with the color reactions of atropine, we may safely conclude, that none of them can be used for quantitative purposes with enough accuracy. Part of them give no color at all with atropine, part of them give a color, which is too unstable to be used colorimetrically.

C. Let us now consider the next group, that of the precipitate reactions. These are also known in great number.

1. *The reaction of Ladenburg*.<sup>11</sup>  $\text{AuCl}_3$  (1:20) gives a faint yellow precipitate in a solution of atropine. When looked at under the microscope, it appears to consist of oily drops. Left to itself, it crystallizes overnight in beautiful small rosettes. Addition of a trace of hydrochloric acid (not too much) accelerates the crystallization. The crystals can now easily be centrifuged into a calibrated capillary tube (as they have first been described by Hamburger in the Biochemische Zeitschrift, 1906, i, 263) and measured. As this method seemed to promise beautiful results, I studied it very carefully, but after some time I saw that it could not be trusted quantitatively, because the salt is still too soluble in water. Until a dilution of about 1:200 was reached, the results were rather satisfactory. One part of the scale appeared to indicate 0.0198 mgm. alkaloid, so that the test is rather sensitive. But in greater dilution—and I always had to determine the amount of atropine in more dilute solutions—it was not to be trusted, and gave very irregular results because of the solubility of the gold-salt. For this reason I gave up this nice method, although I think, that it would be worth while to try to find out means to use it.

2. *The reaction of Duflos*.<sup>12</sup>  $\text{PtCl}_4$  gives an analogous precipitate.

3. *The reagent of Mayer*<sup>13</sup> of Valser,  $\text{KI.HgI}_2$ , and

4. *The reagent of Dragendorff*,<sup>14</sup>  $\text{KI.BiI}_3$ , give gray or brown precipitate with all alkaloids. The sensitiveness of the reaction is greater in acid solution than in a neutral one. The precipitate cannot be determined in the way in which I determined the gold-precipitate, because it adheres to the walls of the vessel, and is not collected at the end of the capillary, neither is it possible to determine indirectly the amount of atropine in determining the mercury, because the precipitate is not constant enough in composition. This was already stated in 1898 by A. B. Prescott,<sup>15</sup> where he says: "The alkaloidal bismuth iodides are not quantitatively uniform enough for alkaloidal assay; but are more stable and less uniform than the alkaloidal mercuric iodides formed by Mayer's reagent. \* \* \* I am well aware how unsatisfactory the latter has been found in the hand of analysts."

There is, however, one way of using this reagent, which I will describe, and which gives rather reliable results. Though this method is still far from being an ideal one, I used it, because a better one could not be found among the 60 or more reagents which I studied for this purpose. My method consists in dividing a part of the watery atropine solution, which I had obtained, as described in the first chapter, into a number of equal parts. When only small quantities of liquid are available, one can simply bring one drop in each of a series of watch-glasses. These equal parts are then diluted with one volume of water, two volumes and so on. Mayer's reagent is now added in equal quantities, and the dilution determined in which the reagent still gives a precipitate. The determination of this limit is very delicate, and it requires some exercise to get the necessary routine. Moreover, the limit is not the same under different conditions of light and for different observers. In my work on the natural immunity of the rabbit for atropine I made for this reason in each series of experiments two control experiments. As the only important information for a physiologist consists in relative data, and the absolute data are mostly rather indifferent, this error is of no great importance. When enough of the solution is available, one can still make an interpolation between the dilution at which he just sees a precipitate, and the next one, though I would not advise this as it lies beyond the limit of accuracy which we can attribute to this method.

An important thing to keep in mind is the fact that according to Kunz<sup>16</sup> the limit is influenced by alcohol, acetic acid, ammonia, glycerine and proteins. It is therefore of the utmost importance, that one be quite sure that the alcohol is quite evaporated, before he takes up the residue in water (see Chapter I), and that one uses fresh sera, in order to avoid the presence of ammonia to any considerable extent. Moreover it is advisable to use exactly the same serum in the control determinations and the experiment. In spite of all these difficulties, I took this method out of the 60 or more mentioned in this paper, because it is the only one which gives approximately reliable results, and is the least objectionable of all.

5. Analogous salts of Ba, Ca, Sr, Cd, Zn, etc. ( $\text{KI.CdI}_2$ , *Marmé's reagent*),

may be used as well as the Hg and Bi salts. An investigation of Herder,<sup>17</sup> however, showed that the sensitiveness increases with the molecular weight of the metal, so that there is no reason to prefer them.

6. *The reagent of Bouchardat*, I in KI, can be used titrimetrically. For this I refer to the next part.

7. The same can be said of *the reagent of Grandeau*, Br in BrK.

8. *The reagent of Hager*,<sup>18</sup> picric acid, is not trustworthy as a reagent as especially the critic of van den Burgh<sup>19</sup> has shown. Though Schaer<sup>20</sup> demonstrated that of the 18 nitro-compounds, which he studied as alkaloid-precipitants, picric acid gave the most reliable results, even these results are not so very satisfactory. Only in etheric solution the picrate precipitates quantitatively, according to Chandelon.<sup>21</sup> In the *Compt. Rendus* 123,929, E. Pozzi-Escot demonstrates, moreover, that picric acid cannot even be used for qualitative analysis.

9. *The reagent of Knorr* (picrolonsäure, i.e., dinitrophenylmethylpyrazolon) cannot be trusted for quantitative work. For the literature see reference 19.

10. Phosphomolybdic acid (*de Vry and Sonnenschein's reagent*), phosphotungstic acid (*Scheibler's reagent*) and phosphoantimonic acid (*Schultze's reagent*), used in their Na salts, might be used in the same way as Mayer's and Dragendorff's reagents. As however the limit of their sensitiveness is lower, our reagent is to be preferred.

11. *Tannic acid* precipitates not only alkaloids, but also proteins, so that it is safer not to use it. Moreover, the results obtained with this reagent are not so very sharp.

12. *The reaction of Veltmann*<sup>22</sup> which has been used in numerous modifications, is based on the precipitation of the atropine with ammonia. As, however, this reaction is not complete, it is better not to use it.

13. The following reactions have been excluded partly because they were mentioned in the literature as absolutely untrustworthy, partly because they do not give any reaction with atropine at all and partly because it is too difficult to obtain them: potassium platinum cyanate; potassium platinum cyanide; metatungstic acid; sodiumnitroferrocyanide ( $\text{Na}_2\text{FeCy}_5\text{NO}$ ); potassium cuprocyanide;  $\text{PbCl}_2$ ; aqueous solutions of Br and Cl:  $\text{K}_2\text{Cr}_2\text{O}_7$ ; potassium  $(\text{Pt}(\text{SCy})_4 \cdot 2\text{KSCy})$ , the so-called salt of *Buckton* (in 4 per cent sol.);  $\text{KSCy}$ ;  $\text{FeCl}_3$ ; silico-tungstic acid; Ir-chloride; Pd-chloride; Na-sulphoarsenate; Na-sulphoantimoniate (the so-called salt of *Schlippe*); boric-tungstic acid; selenosulphuric acid and formaline-sulphuric acid.

D. 1. The *alkalimetric determination* method is as old as the determination problem itself. Schloessing in the *Arch. d. Pharm.*, 1847, ci, 63, was the first to work out a method in this direction. On the question what indicator has to be used opinions are still divergent. I refer to a paper on this subject of E. Runne in the *Apoth. Ztg.*, Berlin, 1909, xxiv, 662. This method is not advisable for our purpose because of small quantities amino acids, carbonates, fats and perhaps phosphates, which may be expected in the liquid. Good methods are those of Elie Falieres<sup>23</sup> and of Elias Elvove.<sup>24</sup>

\*Schultze's reagent gives no reaction with atropine according to the Chem. Kal.



2. A second titrimetric method is the *iodometric method*. Rudolf Wagner, Dingler's Journal, 1861, clxi, 40, was the first to use it. A very good and reliable method has been worked out by Gordon and Prescott<sup>25</sup> which is based on the formation of atropine enneaiodide in very dilute solution. In some previous experiments I could state, that it can be very well adapted to microchemical use; the presence of traces of proteins and fats which absorb the iodine prevents us from using this method for our purpose.

3. Mayer's reagent and that of Dragendorff are sometimes used titrimetrically. Because of the small quantities of atropine our method is, however, to be preferred.

4. For morphine: Its reducing qualities have been used by Kieffer<sup>26</sup> and Reichard.<sup>27</sup>

E. Our last method of determination of alkaloids is a very sensitive one, the capillary-analytical method, in which the stalagmometer of Traube is used. It is, however, so very sensitive, that the smallest quantities of inorganic salts disturb the determination. For this reason it cannot be used for our purposes.<sup>28</sup>

Exclusively qualitative are two odor-reactions on atropine.<sup>29</sup> (Cited after 9.)

1. When atropine is heated with conc.  $\text{H}_2\text{SO}_4$ , it smells like orange-blossom.

2. Heated with conc.  $\text{H}_2\text{SO}_4$  and some crystals of ammonium molybdate, it smells like bitter almonds.

#### SUMMARY

Atropine has to be determined quantitatively in 1 c.c. of serum, containing maximally 5 mgm. alkaloid. In test-tubes of about 15 c.c. the proteins are precipitated with absolute alcohol. The precipitate is washed out several times with alcohol, and does not contain such a quantity of alkaloid as can be demonstrated. The alcoholic solution is evaporated. The residue is taken up in water. This watery solution is concentrated to a few c.c. A precipitate principally consisting of fats is filtered off with a microfilter. The quantity of atropine in this watery solution is determined with the reagent of Mayer,  $\text{KI.HgI}_2$ , which appeared to be the least objectionable of 60 or more tested reagents. The dilution is determined in which this reagent is just capable to give a precipitate. From this the quantity of atropine can easily be calculated. Some sources of error are indicated on p. 284. Besides the experiment one should always make a control determination!

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## THE PHENOMENON OF BACTERIOPHAGE\*

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(Received for publication July 19, 1921.)

**B**ACTERIOPHAGE is a recently recognized phenomenon which is due to the activity of a substance occurring under certain conditions in bacterial cultures which has the property of destroying bacteria. The peculiar properties of this principle have stimulated considerable research in regard to its nature and the possible relation it may have to immunity. The discovery of Bacteriophage or d'Herelle's phenomenon, as it is frequently called, has recently been attributed by Doctor Jules Bordet<sup>1</sup> to F. W. Twort<sup>2</sup> who published an article in *The Lancet*, London, in 1915, describing in detail the process of autolysis which he observed in agar colonies of a micrococcus developing from calf vaccinia. Since 1917 detailed studies of this phenomenon have been made by d'Herelle, by Kabeshima and by Bordet and Ciuea. There have also been contributions by Gratia, Salimbeni, Kuttner, Maisin, Dumas, Elvira and Pozerski, Wollman, Debre and Hagenau, and Bablet.

The conflicting theories to explain this interesting phenomenon are best understood if preceded by a brief description of the facts available. An active lytic substance such as d'Herelle employs<sup>3</sup> is obtained from the feces or urine of a patient suffering from dysentery. An emulsion is made in broth and the material filtered. Bordet and Ciuea,<sup>4</sup> by inoculating a guinea pig intraperitoneally with several doses of *B. Coli* obtained a peritoneal exudate which had this same property of dissolving or inhibiting a culture. This lytic principle was at first thought to be specific for the causative organism but it is now known to be active against a number of different strains and even under certain conditions against a number of related groups of organisms. This lytic property can be transmitted in series indefinitely either from very minute quantities of an inhibited or dissolved culture or from an agar colony which is resistant to the action of the lytic substance. This activity is only demonstrable in young living cultures.

In the first investigations carried out by Twort<sup>2</sup> the phenomenon was observed as a slow lysis of bacterial colonies developing on agar from glycerinated calf vaccinia. It was also found to have some activity on staphylococcus albus and aureus, but not on streptococcus or *B. Coli*. He discussed the possibilities of several theories to explain this lytic activity, namely: (1) The presence of an ameba form; (2) a stage in the life history of the micrococcus which will not grow in ordinary media; (3) a minute bacterium; (4) a still lower form of life (protoplasm without form or an enzyme with the power of growth); (5) an enzyme secreted by the micrococcus. He was inclined to

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believe it is such an enzyme or, he states, it "might rather be accepted as an acute infectious disease of the micrococci." He demonstrated the activity of this substance after passing it through the finest filter and after heating at 52° C. for one hour. He found it maintained its activity for six months on agar but that it would not itself grow on any medium. It was not of an infectious nature.

F. d'Herelle<sup>5</sup> has maintained from the first that the bacteriophage activity is due to a diastatic action of a resistant filterable organism—a bacterium, parasite or protozoan. He has worked principally with *B. dysenteriae* Shiga, using filtrates of a broth suspension of the excreta of dysentery patients. He considers<sup>6</sup> the lytic substance a normal host of the intestines, during health adapted to the destruction of *B. Coli* but in dysentery adapting itself to *B. dysenteriae*. The principle is obtained in any intestinal disease as primarily specific for the causative organism,<sup>7</sup> the activity depending on the virulence of the bacteriophage organism rather than the numbers present. D'Herelle describes an abnormal form in a culture in the process of dissolving<sup>8</sup> which is visible by the use of the ultramicroscope. It is a spherical form supposedly of the organism containing the multiplying bacteriophagic organism. It is seen rupturing and scattering many minute bodies into the medium. This liberates the first generation of the parasitic organism along with a diastase. Ordinary microscopic examination can reveal nothing because—1st, an active filtrate can pass through a collodion membrane which limits molecules of serum albumin, and 2nd, a beam of light does not detect particles in such a solution. He states, however, that there is a sediment on centrifugalization or on standing. The action of physical and chemical agents such as heat, time, ether, glycerine he believes to be compatible with life and cites analogous experiments with organisms. In explaining the action of this bacteriophagic organism, d'Herelle indicates<sup>9</sup> the possibility that a bacillus may acquire an immunity against an infection of such a parasite, and thus would be explained the nature of relapses in typhoid. Immunization, he says,<sup>8</sup> has two phases, the active lysis, and an indirect action that modifies the bacterium. A protection against infection has been demonstrated by the use of a dissolved culture, and in human infections of dysentery five cases were cleared in 24 hours by the ingestion of such a substance.<sup>10</sup> D'Herelle also finds that an antilytic serum is produced by the introduction of bacteriophagic culture filtrates in the animal body. This, he contends,<sup>11</sup> does *not* destroy the bacteriophagic organisms, but merely inhibits them for a length of time depending on the amount of serum used. This antibacteriophage serum also has a powerful antiimmunizing power.

Bordet and Ciueca<sup>4</sup> obtain the lytic principle from a filtrate of a peritoneal exudate produced by inoculating a guinea pig repeatedly with *B. Coli*. They believe it is a phenomenon of defense due to the leucocytes which give to the organism an hereditary property of producing a lytic ferment. This ferment is capable of being diffused in a liquid medium and affecting normal organisms of the same species. Subsequent generations of a lytic strain inherit this new property. The acquired characteristic<sup>12</sup> may be due to a temporary

external factor which breaks the equilibrium between the construction and destruction of living matter. This variation operating for the first time on a bacterium is transmitted by it to its descendants. It is also diffusible in culture media and will cause a new modified race by merely coming in contact with normal bacteria. Therefore the variation may be considered not only hereditary but contagious. Bordet and Cinca<sup>13</sup> also obtained an antilytic serum by immunizing with a dissolved culture or with a strain of *B. coli* obtained from an organism developing in a culture which partially resists the action of a lytic fluid. This antilytic serum they find to inhibit the action of ten times the amount of lytic fluid—in other words, they believe there is a direct neutralization of this substance. The explanation of the persistence of *B. coli* in the intestines in the presence of such a lytic substance is suggested by an experiment in which invulnerable organisms are produced, that is, a strain having no power to lyse a normal culture and not being dissolved itself by a lytic fluid. This organism is produced by the action of a lytic substance on a normal culture of *B. coli* over an extended period of time.

Recent work by André Gratia has been especially directed towards the study of the behavior of organisms resistant to the action of the lytic fluid, in a comparison with sensitive strains. Using a culture of normal *B. coli* he has found<sup>15</sup> that after a culture is partially dissolved the resistant organisms are characterized by (1) enhanced virulence; (2) greater motility; (3) slow growth in broth with a late pellicle formation and a sediment which is not compact, but with a clear supernatant fluid; (4) a diffuse growth in an agar stab; (5) characteristic transparent irregular colonies on agar; (6) in peritoneal infections it produces an exudate of a serous nature. Gratia has also found<sup>16</sup> that a normal culture of *B. coli* which is allowed to become old and dry contains certain organisms which are resistant to the lytic substance. He believes that ageing and the lytic principle both have a selective action rather than an action founded on hereditary adaptation. Gratia has found<sup>17</sup> that the most favorable reaction of a medium for the production of a lytic substance is slightly alkaline, and he has also worked on the specificity of the principle, concluding that by successive passages with allied species of organisms the bacteriophage becomes acclimated and will act as a specific lytic agent. He finds<sup>18</sup> a lytic fluid obtained from a resistant culture of *B. coli* is not as specific as one obtained from a very sensitive culture.

Among other theories of this phenomenon we have that of Kabéshima.<sup>19</sup> He believes the activity of such a lytic substance is due to a "ferment d'immunité bactériolysant" or that there occurs in the glands of the intestines a catalyzer which acts on a prodiastase in the microorganism forming a ferment which is a catalyzer for organisms of a new generation. He especially refutes d'Herelle's theory by showing that the properties of the lytic substance<sup>20</sup> are similar to those of an enzyme rather than of a living organism. For instance he precipitates the active principle with acetone and extracts it with ether. D'Herelle<sup>21</sup> points out that *B. subtilis* may be precipitated by the same method and even after drying resistant forms will persist and develop in broth. He does not find the principle soluble in ether. Kabéshima found as did d'Herelle



that a sediment occurs on centrifugalization of an active filtrate but this sediment he found no more active than the supernatant fluid. D'Herelle finds the greatest resistance to heat exhibited by the lytic principle after it has stood about fifteen days. Heating at 68° C. for one hour immediately after the lysis of a culture is complete, diminishes the lytic power considerably. Thus he shows the similarity to resistant forms of microorganisms. By counting the number of clear zones appearing on agar growths with the addition of a bacteriophage substance d'Herelle computes the number of active individuals per c.c. Then using a very small amount of this highly diluted in ten tubes of a *B. dysenteriae* Shiga culture he found lysis occurred in three of the tubes. This he took as an indication<sup>22</sup> that there were present active individuals rather than a diffusible enzyme.

Salimbeni claims to have found a myxameba in lytic filtrates which he has named "myxomyces shigaphagus."<sup>23</sup>

J. Dumas<sup>24</sup> has reported the presence of a bacteriophagic substance active against *B. coli* and *B. dysenteriae* Shiga, but not against cholera, in normal feces, soil, city water, Seine river water and conduits. He finds the presence of broth is necessary to produce a lytic fluid. In some relation to this last fact, perhaps, Miss Kuttner<sup>25</sup> notes that in a cleared culture if the tube is reclouded, fresh broth must be added before more of the lytic principle will be active.

As can be seen from this brief survey of the literature, the interest in this phenomenon lies principally in solving the problem of the nature of the active principle and its function in infection and immunity. So far the problem has only been broached and its ultimate significance, although undoubtedly considerable, lies in the future.

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# LABORATORY METHODS

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## GLUCOSE TOLERANCE TEST\*

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### DEFINITION

THE term "glucose tolerance test," or better, "blood sugar tolerance test," is applied to the series of blood sugar determinations following the ingestion of one hundred grams of glucose, showing what is known as the sugar tolerance curve.

In the average normal individual during the period of nonabsorption from the gastrointestinal tract the amount of dextrose in the blood remains constant between the limits of sixty and one hundred twenty milligrams per 100 c.c. of blood. During the period of absorption this amount is considerably increased—the postprandial hyperglycemia. Following the ingestion of one hundred grams of glucose the concentration of sugar in the blood begins to increase, reaching a maximum of not to exceed one hundred sixty milligrams at the end of forty-five minutes to one hour, and soon begins to decline rapidly, reaching approximately the original concentration by the end of two hours. This is known as the normal curve. (Solid line, Fig. 1.)

Macleod<sup>1</sup> states that during the period of nonabsorption the concentration of sugar in the portal vein is approximately the same as that in the systemic circulation, but during the period of absorption that of the portal circulation is considerably higher. The liver, interposed as a barrier between the two, has been proved capable of elaborating and storing glycogen to the amount of one hundred fifty grams; the muscles also are able to remove from the circulating blood and store an equal amount. The glycogen thus stored in the muscles constitutes the immediate supply of carbohydrate for body oxidation, that in the liver the reserve supply. But the liver and muscles are not able to care for all the absorbed sugar immediately, but allow some to pass into the general circulation, hence the temporary hyperglycemia following the ingestion of carbohydrates. The determination of the height and persistence of this postprandial hyperglycemia constitutes the tolerance test.

### CONTROL OF CARBOHYDRATE METABOLISM

At every muscular contraction glucose is burned up and the reserve supply in the liver is drawn upon by a process of hydrolysis, through the action of an enzyme, glycogenase, either in the liver cells or in the circulating blood and lymph, the process being known as glycogenolysis. The mechanism

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\*From the Clinical Laboratories of the State University School of Medicine. Read before the Oklahoma County Medical Society April 9, 1921.

of this process is under control of the endocrine secretions, and is so finely adjusted that excessive concentration in the blood does not occur.

This adjustment is brought about to a limited extent by nervous control, as is evidenced by the so-called nervous glycosurias, the experimental glycosurias caused by stimulation of the splanchnic nerves, and the glycosuria following injury to the floor of the fourth ventricle. This control, however, is probably only secondary, due to hyperactivity of the suprarenal glands, from stimulation through the sympathetic system (McKenzie<sup>2</sup>). But for the most part the mechanism is under control of the internal secretions direct.

With reference to the process of glycogenolysis the internal secretions have been classified as (a) Inhibitors of glycogenolysis, and (b) Accelerators of glycogenolysis (Tidy<sup>3</sup>). Because of the accurate equilibrium of these two opposing forces, the sugar content of the blood is maintained at a constant level; if either becomes deranged the equilibrium is lost, and a hyperglycemia or a hypoglycemia results.

The secretion of the suprarenals is the most important of the accelerators of glycogenolysis. The hyperglycemia of depancreatized animals rapidly drops after removal of the suprarenals (McKenzie<sup>2</sup>); the injection of adrenalin will cause a hyperglycemia and glycosuria; in fact, Rhodenberg, Bernhard and Krebiel found the tolerance test can be carried out equally as well by the injection of one cubic centimeter of a  $\frac{1}{1000}$  adrenalin solution as with the ingestion of one hundred grams of glucose. So in hyperactivity of this gland one will find a high blood sugar curve indicating a low carbohydrate tolerance. On the contrary a low sugar concentration and high tolerance is found in Addison's disease. The thyroid plays an important part as an accelerator of glycogenolysis, consequently in hyperthyroidism there is a hyperglycemia, while in cretinism and myxedema the opposite is true. The pituitary body is also an accelerator, and hyperglycemia and glycosuria are more or less constant in the early stages of acromegaly. These secretions probably inhibit the process of glycogenesis as well as accelerate the process of glycogenolysis.

On the other hand the internal secretion of the pancreas plays a most important part as an inhibitor of glycogenolysis, and is the main factor in the process of glycogenesis; and it is in deficiencies of this secretion that we find the most marked and most typical hyperglycemia and glycosuria, that of diabetes mellitus. The parathyroids play but a minor part as an inhibitor of glycogenolysis.

#### CARCINOMA AND CARBOHYDRATE METABOLISM

In addition to the endocrine disturbances mentioned there are a number of other conditions that disturb carbohydrate metabolism, notably tuberculosis and carcinoma, which appear to act very much as the accelerators of glycogenolysis.

Friedenwald and Grove<sup>5</sup> in summarizing the work on blood sugar determinations call attention to the statements of Freund and of Trinkler that there is always a hyperglycemia in carcinoma, a statement which we have not been able to substantiate. Many observers have noted the low tolerance to dextrose in these patients, and Rhodenberg, Bernhard and Krebiel<sup>4</sup> in 1919 and



1920<sup>6</sup> made extensive observations on nearly three hundred cases. In their preliminary report they described a very definite curve which they called the carcinoma curve. This occurred in twenty-four cases of carcinoma and one of sarcoma, and did not occur in forty cases of other diseases; in their second report<sup>6</sup> they found the same curve in certain other conditions, and some cases of proved carcinoma did not give the typical curve.

Friedenwald and Grove<sup>5</sup> reported studies in a series of thirty-two cases of gastrointestinal carcinoma in which they observed what they believe to be a rather characteristic curve, and which was not found in any of fifty-five other cases including five cases of extragastrointestinal cancer. While they have not had the opportunity to study early cases of gastrointestinal carcinoma, they are of opinion that the test will prove quite as definite in early cases as in late ones, and therefore will be of great value in early diagnosis.

Edwards<sup>7</sup> in 1919 reported the use of this test in twenty-nine cases of cancer, and found the curve reaches the neighborhood of two hundred milligrams at the end of forty-five minutes, and at the end of two hours is at approximately the same level, or may be considerably higher, than at the forty-five minute period. He concludes that failure to establish a "cancer" curve in the test is strong evidence against malignancy.

#### PERSONAL OBSERVATIONS

Our series of cases consists of 225 observations on 154 patients, fifty-three of whom had proved carcinoma. Our results correspond fairly closely with those of the workers just cited.

The test has been carried out as follows: Five cubic centimeters of blood is taken in the morning before the patient has had anything to eat; one hundred grams of anhydrous glucose is then given, dissolved in a cup of coffee; forty-five minutes from the time the glucose is taken five cubic centimeters of blood is drawn, and again at the end of two hours from the beginning. The amount of dextrose is then determined by the Folin-Wu<sup>8</sup> method. This consists essentially in the complete removal of the proteins from the diluted blood, reduction of a copper solution by the sugar in the filtrate, development of an intense blue color by a reaction between the reduced copper and a phosphomolybdate-phosphotungstate solution, and a comparison of this color with a standard dextrose solution similarly treated.

The results can be grouped in three fairly distinct classes as in the work of the previous observers.

Type 1 curve begins within or above the normal limits for a fasting individual, rises sharply to near two hundred milligrams at the end of forty-five minutes, and drops slightly, remains at the same level, or rises above the forty-five minute figure at the end of two hours. (Broken line, Fig. 1.) A certain number of atypical cases occur. These have the general characteristics of this curve except in that the rise is not so sharp or so high as in the more pronounced cases.

Type 2 curve, the normal curve, begins within normal limits, rises somewhat more gradually than does Type 1, and at the end of two hours has returned to near, or even below the original figure. (Solid line, Fig. 1).

Type 3 curve begins within normal limits, does not rise above the initial figure, and at the end of the test period is as low or lower than in the beginning. This is the type of curve we should expect to find in hypoactivity of the thyroid, suprarenals and pituitary glands. (Dotted line, Fig. 1.)

#### COMMENT

Table I shows the results of the test in 127 cases.

Factors affecting absorption from the intestinal tract have not been taken into consideration. Such factors may be responsible for the negative results in gastric carcinoma, as those cases giving Type 1 atypical curves were advanced cases with extensive involvement of the stomach.

Two cases of hyperthyroidism and two of hypothyroidism are tabulated. The results are exactly what we should expect. Four cases of neurasthenia are shown, all giving a curve exactly like that of hyperthyroidism. In neurasthenia there is hyperactivity of the thyroid. (Bandler.<sup>9</sup>)

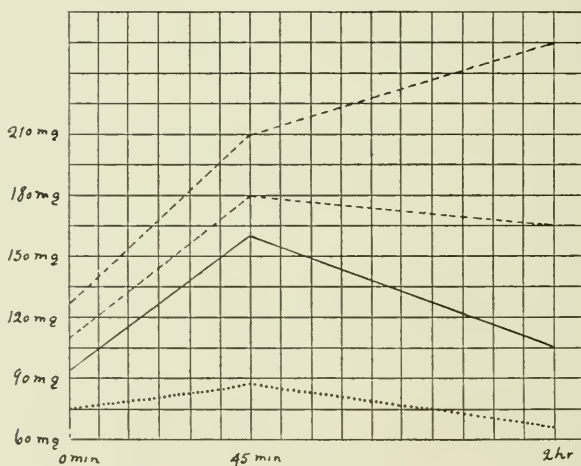


Fig. 1.—Broken line Type I, solid line Type II, dotted line Type III.

Diabetes mellitus invariably gives a Type 1 curve, and is remarkable for the height to which the blood sugar rises.

Of seven cases of sarcoma, three give a Type 1, four a Type 2 curve.

Of four cases of aortic aneurysm, all having a positive Wassermann, three give a Type 1 curve, in contrast to a number of other cases of syphilis, all of which give a Type 2 curve.

Of seven cases of jaundice from various causes, five, or 71 per cent show a Type 1 curve; therefore the test is of no value in suspected carcinoma complicated by jaundice.

Twenty-nine cases of tuberculosis are shown. Twenty, or 60 per cent show a Type 1 curve. Three out of six cases of very early incipient tuberculosis, or 50 per cent give this reaction. In one case, that of a young woman (worker in the laboratory) this was the first indication that she was abnormal. In running the test on a series of apparently normal individuals, this person was found to have a typical Type 1 curve. A few weeks later, when she

TABLE I

	TYPE 1 CURVE	TYPE 1 ATYPICAL CURVE	TYPE 2 CURVE	TYPE 3 CURVE
Carcinoma, Superficial	10	1		
Carcinoma, Gastric	3	3	2	
Carcinoma of Liver and Gall bladder	2	1		
Carcinoma of Colon	7			
Carcinoma of Rectum	4			
Carcinoma of Bladder	5			
Carcinoma of Prostate	5	1		
Carcinoma of Breast	2			
Carcinoma of Vagina	2			
Carcinoma of Cervix and Uterus	3		2	
Sarcoma, Various types	3		4	
Tuberculosis, Incipient pulmonary	3		3	
Tuberculosis, Advanced pulmonary	14	2	3	
Tuberculosis, Other than pulmonary	3	1		
Jaundice due to causes other than malignancy	5		2	
Osteomyelitis, Chronic	6	1		
Diabetes Mellitus	4			
Neurasthenia	4			
Hyperthyroid	2			
Hypothyroid				2
Gout				1
Hypertension, Essential				1
Obesity	1	1		
Cirrhosis of Liver	1		1	
Pernicious Anemia	1		1	
Aneurysm of Aorta	3		1	

called attention to her run down condition, tubercle bacilli were found in great numbers in her sputum.

Fifty-three cases of carcinoma are reported. These are all proved cases, either by tissue examination, postmortem or by unmistakable clinical signs. It will be noted that forty-three cases or 81 per cent give a typical Type 1 curve, (exactly the same type of reaction as is found in cases of hyperactivity of the thyroid, suprarenals, etc.), while six give an atypical Type 1 curve, and only four a Type 2. It is significant that in many cases there was a marked change in the type of the curve following removal of the tumor, in a few cases reaching normal within a month. In some cases, tissue examination revealed a very early malignancy, but the sugar curve was typical. This is significant.

Repeated tests have been run on numerous cases. The results fluctuate considerably, but invariably, the type is the same.

Carcinoma of the stomach gives the largest percentage of atypical Type 1 curves. In all these cases there is either extensive involvement of the stomach or obstruction of the pylorus with resulting retention of the sugar in the stomach; consequently the slow absorption and the low curve.

We have found only Type 2 reactions in some twenty other conditions not tabulated, including spastic colitis, ileocolitis, mastitis, lymphatic leucemia, hypertrophy of prostate, prostatic abscess, orchitis, urethral stricture with retention, acute tonsillitis, pancreatitis, salpingitis, malaria, dementia precox, acute articular rheumatism, arthritis deformans, obliterative arteritis, and syphilis.

A large number of determinations on normal individuals have given only Type 2 reactions.

Two explanations for the altered carbohydrate metabolism in carcinoma have been offered by Dr. Simon, quoted by Friedenwald and Grove<sup>5</sup>: 1, The increased demand of the growing tumor for carbohydrate; and 2, A hormone liberated by the carcinoma cells impairing the power of the tissues to oxidize sugar. We would suggest a third possibility, that a specific hormone is liberated by the carcinoma cells which acts to accelerate the process of glycogenolysis or retard the process of glycogenesis, or both, either directly, or by acting upon one or more of the endocrine organs or the vegetative nervous system.

#### CONCLUSIONS

Our series of cases is too small to form the basis of definite opinion. From a study of these results and of the literature we arrive at the following conclusions.

(1) Carbohydrate metabolism is under control of the internal secretions, and is sensitive to changes in endocrine function. Therefore blood sugar determinations may aid in the diagnosis of these conditions.

(2) Carbohydrate metabolism is disturbed by carcinomatous growth, apparently in the same way as in certain endocrine disturbances.

(3) This disturbance is probably due to a secretion of the tumor cells; consequently the effect may occur quite early.

(4) If this be true, the blood sugar tolerance test should be a valuable diagnostic test.

(5) There is no sugar tolerance curve definitely characteristic of carcinoma, but most cases of carcinoma give a certain type of curve which is found in comparatively few other conditions, including tuberculosis, diabetes mellitus and hyperthyroidism.

(6) The test is of no value in suspected carcinoma of the bile tract area complicated by jaundice.

(7) The test needs further confirmation.

I wish to acknowledge my indebtedness to the technicians in the Clinical Laboratories for their efficient work, and to the Staff of the University Hospital for the privilege of studying their cases.

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## AN IMPROVED METHOD OF CAGING AND FEEDING MICE\*

BY HELEN S. MITCHELL, NEW HAVEN, CONN.

ALTHOUGH the caging and feeding of mice is a common laboratory procedure, a particularly convenient form of individual mouse cage which facilitates handling and makes possible an accurate determination of food intake may be of interest to other workers in this line.

The wire cage without the special feeding device has previously been used by Wheeler<sup>1</sup> in this laboratory. Views of the separate parts and the assembled cage are shown in the accompanying photographs. In the top a trap door large enough to admit a person's hand makes it possible to introduce material into the cage without a chance for the animals to escape. A de-

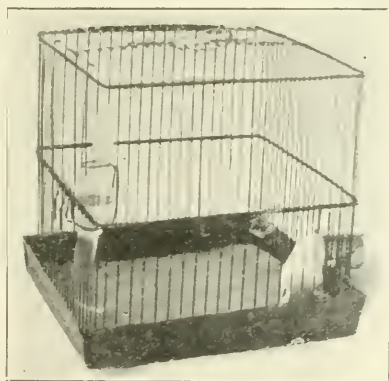


Fig. 1.—Showing the experimental cage and feeding tube in use.

tached shallow pan in which is inserted a sheet of absorbent paper covered by a corresponding square of fine wire gauze, forms the floor of the cage and can be easily cleaned and sterilized. The paper serves to retain the urine voided and the wire gauze prevents the animals from tearing or gnawing the paper.

Most investigators have experienced difficulties in feeding mice because these animals scatter their food and thus prevent an accurate determination of food intake. The use of a paste food which has been found advantageous for rats was unsatisfactory in the case of mice which invariably pawed the whole cup-full out onto the floor of the cage. To avert such spilling of food a very simple device was suggested by the more elaborate outfit for rat-feeding described by Hopkins and Ackroyd<sup>2</sup> and Macallum.<sup>3</sup> This device was easily attached to our wire cages and proved most satisfactory in preventing scattering and in keeping the food free from urine and feces.

\*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.  
Received for publication June 23, 1921.

An elbow of  $\frac{3}{4}$  inch tin pipe was cut to such a length that the horizontal section extended out over the edge of the pan and the vertical part reached down into a porcelain crucible used as food cup. A vertical cage wire was cut to permit the insertion of the feeding tube. The bent ends of the wire and the flanges on the tube prevented the dislodgment of the latter. The paste food tended to hold both the cup and feeding tube firmly in place and thus hindered

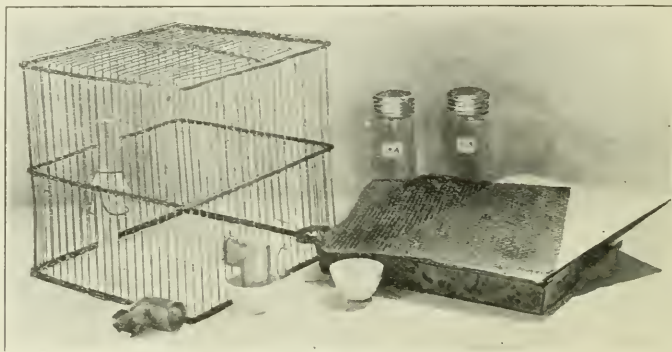


Fig. 2.—Showing the individual parts of the mouse cages and the feeding devices used for experimental animals.

the mouse from pushing the cup away and escaping. To make the device most successful it was found advisable to make the food in the form of a firm paste and to keep the cups well filled.

The expense of elaborating these cages was defrayed by a grant from the Russell H. Chittenden Research Fund for Physiological Chemistry. They were manufactured by the Andrew B. Hendryx Co., New Haven, Conn.

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# The Journal of Laboratory and Clinical Medicine

VOL. VII.

FEBRUARY, 1922

No. 5

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Washington, D. C.

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## EDITORIALS

### *Basal Metabolism*

THE determination of the basal metabolic rate in routine clinical work is now possible, even for the private patient at home. New terms have come into use which the general practitioner is expected to know and use. An attempt will be made to explain the meaning of these terms and to describe briefly the principles underlying the determination of the basal metabolic rate.

The body is made up of cells, each of which must carry on and take its place in the performance of body functions. There are certain processes, chemical and physicochemical, going on within the cell all the time and upon these processes the life of the cell depends.

It is necessary for a city to have an influx of food and fuel and also to have an adequate waste disposal system; so the individual, a city of cells, must have a supply of food and fuel carried to the cells by the blood and a waste disposal system, again the blood stream, to carry the waste products  $\text{CO}_2$ , urea, etc., to the disposal stations, the kidneys, lungs and skin.

The cells take up the food brought to them by the blood, synthesize the

food elements into their own structure. The cells themselves are then oxidized, giving off carbon dioxide and water. To these chemical and physico-chemical processes going on within the cells has been applied the term "metabolism." This term, in the original, means "change" and in the dictionary it is defined as that process by which living cells incorporate the matters obtained from food into their own structure. The rather limited normal metabolism of the body cells is dependent on an adequate supply of oxygen and the adequate removal of the carbon dioxide.

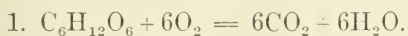
The amount of metabolism is affected by three groups of processes. These are, 1st. The vital processes such as respiration and circulation. 2nd. Digestion. 3rd. Muscular exertion. The amount of the metabolism is the least when the latter two factors are eliminated as far as possible allowing the first group to reach a minimum. This low line of metabolism is spoken of as "basal" metabolism. It is obtained by having the patient abstain from eating or drinking anything other than water for twelve hours or more and by requiring him to lie quietly for at least thirty minutes prior to performing the test for the determination of the basal metabolic rate. This rate is usually expressed in the number of heat units or calories produced by the individual per hour per square meter of body surface. The latter is determined from the nude weight and height of the patient. This rate may then be compared with the rates which have been determined for a large series of healthy individuals of both sexes and of varying ages, and the result of the comparison is expressed as percentage above or below the normal. Eighty-six per cent of all normal persons have a basal metabolic rate which is within 10 per cent of the curve for normals.

There are two principal methods of determining the metabolic rate; direct and indirect calorimetry, the one depending on the direct measurement of the heat loss of the individual, the other being computed from the oxygen consumption, the carbon dioxide liberated, and the nitrogen output in the urine.

The two methods are combined in the large respiration calorimeters of the Sage Foundation, the Mayo Clinic, and other large institutions. Briefly this calorimeter consists of a closed room surrounded by water, the weight and temperature of which is known throughout the experiment and from this the amount of the heat loss from the patient is measured directly. While in the calorimeter the patient is supplied with air having a definite oxygen content. By means of a powerful blower or fan the air is kept in circulation and is forced from the room through sulphuric acid which removes the moisture, then through soda-lime which removes the carbon dioxide, and then through another bottle of sulphuric acid to remove the moisture absorbed from the soda-lime. By weighing accurately these different substances before and after the experiment one can determine the exact amount of the oxygen used, the amount of moisture and the amount of carbon dioxide liberated by the individual. The ratio of the volume of the  $\text{CO}_2$  liberated to the volume of the O absorbed is known as the respiratory quotient and its value depends upon the type of food which is being consumed or metabolized by the body cells. Thus



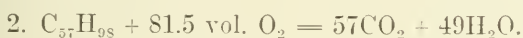
for carbohydrates the volume of the  $\text{CO}_2$  liberated by their oxidation is the same as the volume of O consumed and the respiratory quotient is 1.



2. 6 volumes of  $\text{CO}_2$  divided by 6 volumes of O equals 1. While for fats there is not enough oxygen in the molecule to form water hence some of the oxygen used is for that purpose, resulting in the formation of fewer volumes of carbon dioxide than of oxygen used. Taking tristearine as an example:—



To form carbon dioxide and water from the  $\text{C}_{57}\text{H}_{98}$  will require 81.5 volumes of oxygen.



$$3. \frac{57 \text{ vol. CO}_2}{81.5 \text{ vol. O}_2} = 0.7 \text{ the r.q. (respiratory quotient) for fat.}$$

For proteins, on the average, the respiratory quotient is about 0.81. Knowing the respiratory quotient one can determine which of the food elements is being metabolized. Each gram of protein or carbohydrate when oxidized liberates 4 calories of heat, while fat liberates about 9 calories per gram burned. The amount of these food elements which may be oxidized by a liter of oxygen has been determined and from this the caloric value may be found (if the r.q. is known). This figure, the caloric value of 1 liter, multiplied by the number of liters used and divided by the surface area of the patient will give the number of calories per hour per square meter of body surface, and is known as the basal metabolic rate. This latter method of indirectly determining the rate from the oxygen and the carbon dioxide requires relatively less complicated apparatus than the respiration calorimeter.

The two methods above described, direct and indirect calorimetry, were found to agree very closely, and this has led to the development of smaller machines for the indirect determination of the basal metabolic rate. These smaller machines are of two types, the one determining both the oxygen intake and the carbon dioxide output and from these findings determining the r.q. and the resulting caloric value of the oxygen used. The other type of machine determines only the oxygen consumption and, using a constant r.q. of 0.82, one may determine the caloric value of the oxygen used. The determinations of the basal metabolic rates on the same individual at the same time with these various types of metabolimeters agree within 5 per cent. and are therefore sufficiently accurate for clinical purposes. This is especially true if the determinations are made by one who is entirely cognizant of the difficulties and of the large "personal equation" which necessarily enters into such determinations.

For persons in health, the normal basal metabolic rates figured in calories per hour per square meter of body surface have been figured out by Aub and Dubois and are as follows:—

AGE	MALE	FEMALE	AGE	MALE	FEMALE
6-8	58	58	20-30	39.5	37.5
8-10	54	54	30-40	38.5	36.5
10-12	52	50	40-50	38.5	36.0
12-14	50	46	50-60	37.5	35
14-16	46	43	60-70	36.5	34
16-18	43	40	70-80	35.5	33
18-20	41	38			

Variations of more than ten per cent from the above findings are considered pathologic. Variations of less than ten per cent are considered physiologic.

With relation to basal metabolic rate there are three classes of disease, i.e.—1st. Those diseases having a normal basal metabolic rate. 2nd. Those diseases having an increased basal metabolic rate. 3rd. Those diseases having a decreased basal metabolic rate. The first class includes those afebrile diseases not of endocrine origin, as, for example, neurasthenia, the nephritides, and cardiac cases which are still in a state of compensation. The second class includes all febrile diseases, those diseases of the endocrine system that are characterized by an increased secretion of the gland at fault, the leukemias, pernicious anemia, paralysis agitans, dyspneic cardiorenal cases, and those cases of diabetes which have an increased protein metabolism. The third class referred to is composed, for the most part, of cases in which there is a decrease in the quantity or quality of the secretion of one or more of the endocrine glands. Cases of cretinism and of myxedema come in this class. Cases of undernutrition will also often show a slight lowering of the basal metabolic rate.

The amount of the increase of the rate in the diseases of the second group may be used as an index to the severity of the disturbance. After ruling out fever as a cause of the increased rate about 90 per cent of all the marked increases found are due to hypersecretion by the thyroid gland. These cases may be divided into two groups, the adenomas of the thyroid with hyperthyroidism and the hyperplastic thyroids with hyperthyroidism. Those of the latter group come on at an earlier age, have a more acute onset, and as a rule have a higher basal metabolic rate. From the standpoint of the basal metabolic rate hyperthyroidism may be grouped into the very severe with an increase of 75 per cent or more, moderately severe having an increase of between 50 per cent and 75 per cent, and mild, including cases having an increased rate up to 50 per cent. Hypersecretion of the posterior lobe of the hypophysis will also produce a marked increase of the basal metabolism while the bilobar disturbance of Fröhlich's disease usually does not cause a change of the basal rate from the normal.

In leukemia the basal metabolic rate may be increased to 40 per cent or 50 per cent with but little clinical evidence of the disturbance of metabolism. It is worth noting that these cases do not show an improvement of the rate

after therapy (such as x-ray of the spleen and the long bones) which causes a marked decrease in the number of pathologic leucocytes. In pernicious anemia the basal metabolic rate may be normal or it may be increased up to 30 per cent. The explanation which has been offered for the increased basal metabolism in leukemia and in pernicious anemia is the large and continuous increase of embryonic white and red cells.

Paralysis agitans alone may give an increased basal metabolic rate up to 50 per cent. In these cases the rise in oxygen consumption is most probably due to the constant muscular tremor with a consequent oxidation of muscle substance. When a case has both paralysis agitans and hyperthyroid disturbance the basal metabolic rate may be very high. One such case coming under our observation showed an increase of 121 per cent. This case was reported as markedly improved following x-ray treatment of the thyroid gland.

Cardiorenal cases which have reached the stage of decompensation may, with the labored breathing, cause an increase of the basal metabolic rate up to 50 per cent.

Diabetic cases may show a normal, an increased, or even in some cases a decreased basal metabolism. The increase usually accompanies an increase in the metabolism of the body proteins.

Those cases showing decreased metabolic rates usually have a definite clinical picture which points toward the diagnosis. The thyroid and the pituitary glands are the glands most often at fault. Snell and others have reported a series of 13 cases of cretinism and myxedema. These cases showed rates varying from -7 per cent to -26 per cent. Sandiford has also reported several cases, one of which had a basal metabolic rate of 40 per cent below the normal. Thyroid therapy brings the rate up to normal in many of these cases but as the rate increases many of them show all the signs of hyperthyroidism even before the rate reaches normal. Undernutrition usually does not cause a marked decrease of the basal metabolic rate.

One uses a thermometer to determine the rise and decline of a fever temperature. One should use the metabolimeter to determine the extent of the change in the basal metabolic rate and to note the effects of treatment whether that treatment be medical, x-ray, or surgical. Used in this manner it is one of the most valuable additions for the study of diseases of endocrine origin.

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—H. L. C. (*per P. G. W.*)

*Intimate Exposure and Contagion in Tuberculosis*

INORDINATELY difficult to estimate is the relative importance of heredity and family exposure in propagating tuberculosis.

It is pretty clear, in spite of an occasional dissenting voice, that the children of the tuberculous too often develop the disease. Our records at Cragmore Sanatorium, in Colorado Springs, show a known and acknowledged positive family history in over 50 per cent of the cases.<sup>1</sup> We very often learn of the disease pursuing a family through three or more generations, and this among intelligent and careful people living in the best circumstances. Not infrequently investigation discloses that a parent or grandparent is an unsuspected chronic disseminator of tubercle bacilli.

Adams,<sup>2</sup> in an endeavor to show that family tuberculosis is not common, reports the family history negative in 60 per cent of cases—that is, positive in 40 per cent. Solis-Cohen<sup>3</sup> speaks of securing positive family histories in a little more than 40 per cent of a group of patients.

If an accurate general census could be made of all persons, regardless of the state of their health, approximately one in five, or 20 per cent, should give a history of tuberculosis in at least one parent, since about one person in ten dies of the disease. The additional percentage of those who are known to have tuberculosis, but recover and die from other causes, is small. The estimate may therefore fairly be based on the mortality percentage, doubled for the two parents. The figure would be increased by including other relatives besides parents, but is in reality reduced by lack of knowledge and lack of candor in our patients, so that it is likely that the latter source of error more than balances the former. As a rough estimate, 20 per cent may be taken as the approximate expectation of a family history of tuberculosis in the population at large.

We have then among the tuberculous a percentage of positive family histories more than double the normal expectation. The family grouping of the disease is striking. Illustrations of this are abundant, and some excellent ones are given by McBrayer<sup>4</sup> in a report of tuberculosis in a village in North Carolina.

But, granted the occurrence of family tuberculosis, how are we to interpret it? Do the children of those whose resistance to tuberculosis has been inadequate inherit a faulty resistance, or is it merely a matter of excessively large and frequent infection, or do both factors operate? Adams, and King, whom he quotes, feel that far from having a lower resistance, persons with positive family histories have a higher resistance, a greater tendency to recovery and chronicity, than others who contract tuberculosis have. There may be truth in this observation, if only adult victims of phthisis are considered, for the adult tuberculous invalid with a positive family history is often a survivor, many of whose less resistant brothers and sisters have already died of acute tuberculosis in childhood.

Against the hypothesis of special susceptibility inherited from tuberculous parents stand the reports of the Oeuvre Graneher.<sup>5</sup> This institution, which removes the children of the tuberculous from their parents and cares



for them in healthful surroundings, reports a very low incidence of tuberculosis among its charges. An identical observation has been made by Calmette<sup>6</sup> in cattle. The low tuberculosis mortality among the native population of Colorado Springs, many of whom are the children of tuberculous parents, has been emphasized by Gardiner,<sup>7</sup> and is evidence to the same effect. For equipoise, we ought to have reports of children from healthy families who have been brought up in close contact with the tuberculous. This often happens, and some cases of resulting disease have been reported,<sup>6</sup> but no large body of data has been collected.

We agree with the conventional creed of contemporary phthisiology in believing early exposure to be of the gravest importance, though we believe also that there are very great and significant racial and individual variations in the power to cope with the disease, and that these appear very early in life—a subject which calls for more ample discussion. Raymond Pearl,<sup>8</sup> one of the foremost students of this problem, says, “Familial contact with active open cases is beyond question a factor in determining the incidence rate of clinically active tuberculosis. It appears equally obvious, however, that it certainly does not account for the whole, and probably accounts only for a small part, of the increase in the incidence of the disease which we find to occur as the amount of tuberculosis in the immediate direct ancestry increases.”

Allowing that intimate exposure in infancy and childhood is likely to be disastrous, there is the still more debated question as to the possible danger of intimate exposure in adult life.

Marriage is of course the most intimate of adult relationships, and the lack of unanimity as to the occurrence of marital contagion in tuberculosis seems to be evidence that this is, on the whole, not so very frequent. Here again there are many complicating and confusing factors. Special emphasis has been laid on the tendency of similar persons to select each other—the “assortive mating” of Karl Pearson. This, however, is flatly denied by Ward,<sup>9</sup> whose numerous communications all present data in favor of frequent adult contagion. Ward thinks that people with tuberculosis are most likely to marry individuals of entirely different type.

Lawrason Brown<sup>10</sup> has indicated one of the reasons for our confusion on this subject, namely the fact that many of the reports dealing with the matter are not based on the whole life history of the exposed mates, and their final mortality, but on the incidence of the disease in a short period of observation. An investigation of his own, in which this error was eliminated, covered forty thousand couples and led to the conclusion that there was an excessive incidence of tuberculosis among the marital partners of the tuberculous, but that possibly two-thirds of this was due to assortive mating. Brown himself, however, believes that adult contagion is important.

Barnes<sup>11</sup> has recently reported that “the histories of 229 consecutive widowed patients admitted to the Rhode Island State Sanatorium 1905-1921, show that 93, or 40 per cent, lost their consorts by death from tuberculosis, a

tuberculosis mortality over three times that of the married people of the community.”

Crouch, at the Modern Woodmen Sanatorium in Colorado Springs, found that 58 per cent of 233 widower patients had lost their wives through tuberculosis. Minnig,<sup>12</sup> who quotes these figures, gives 50 per cent as his own observation.

Against these positive reports we must weigh the great body of statistics from hospitals and sanatoria, showing a lack of any excess tuberculosis morbidity and mortality among those who care for the tuberculous. Here, however, the contact is not very intimate—at least it does not combine intimacy with perpetuity as marriage does.

Perhaps the various statistical researches which are now under way will illuminate this dark and disputatious corner of our ignorance. At present one feels that expert opinion, which changes so often, is moving in the direction of more emphasis on the possible danger of very intimate and prolonged exposure to the tuberculous invalid, even in adult life, and this in spite of much erudite argument to the contrary.

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G. B. W.—(C. T. R.)

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### *Sputum Examination*

**S**PECIMENS of sputum are examined less carefully, as a rule, than any other material from the body, possibly excepting the feces, and yet a considerable amount of information can be obtained from the study of it, provided the specimens are properly obtained. Too frequently specimens are mixtures of buccal, pharyngeal and nasopharyngeal secretions with some admixture of bronchial material. Examination of such mixed specimens is, if not valueless from a pulmonary standpoint, at least misleading.

On the other hand studies of sputum raised by a true pulmonary coughing act may give information which, taken together with x-ray studies of chest plates, is exceedingly valuable for diagnosis, prognosis, and treatment.

There is no need to recount the value of sputum examinations in tuberculosis, except to note that modern methods of digestion of the material with alkali followed by centrifugalization (Raphael and Eldridge) gives more accurate results than the older method of merely smearing the material on slides.

In asthma too, very useful information may be obtained in making a differential diagnosis.

Asthma may be of several origins. It may be a disease *sui generis*. It may be a symptom of renal, or cardiac, or pulmonary disease. The characteristic laboratory finding in true bronchial asthma is eosinophilia in the sputum, and this according to Bezanceon and Dr. Jong has almost as much value in the diagnosis of asthma as the finding of tubercle bacilli in the diagnosis of tuberculosis. According to these writers Charcot-Leyden crystals, and Curschmann's spirals are of little significance. Also they say the sputum in true asthma is free from albumin which appears only in complicated cases. It is especially in men over 50 who present attacks of nocturnal dyspnea of an asthmatic character that sputum examinations have a special value in determining whether the disease is true asthma, or whether it is a cardiovascular affair.

It is not, however, only in tuberculosis and asthma that microscopic study of the sputum is of real diagnostic value. In bronchial spirochetosis, for instance, a disease which in many respects resembles influenzal bronchitis, certain types of pneumonia and tuberculosis, the diagnosis can only be established by careful study of the sputum. Mason has recently reported a case of this infection in which the spirochetes were found in a pleural empyema. Rapid improvement followed intravenous salvarsan. Pulmonary streptothricosis is another disease which, though it may resemble clinically other types of lung infection, for instance tuberculosis, is due to the presence of filamentous organisms which show true branching. Such cases are sometimes referred to as pseudotuberculosis. Bridge in California has reported fifteen cases of pulmonary streptothricosis. In some of his cases the streptothrix was associated with the tubercle bacillus. In one of Zenoni and Macchi's cases there was a pure streptothrix infection. In pure cases there was no tendency for the disease to begin in the apex of the lung as in tuberculosis; the fever was rarely high, and emaciation was not rapid. Hamman says of these cases that the clinical symptoms are those of tuberculosis except for the localization in the lower lobes. In such cases both sputum examinations and radiograms have a place.

Occasionally certain species of molds attack the lungs, most frequently that known as *aspergillus fumigatus*. Usually it is a secondary invader in tuberculous cases, but as Hamman says it may affect the lungs primarily causing ulcerative and suppurating lesions. *Aspergillus fumigatus* is widely distributed in the outer world and may be found on vegetables, grain bread, and other food stuffs. It is presumed that infection follows inhalation of the spores. There is nothing characteristic in the clinical picture of aspergillosis, and a diagnosis can only be made by microscopic examination of the sputum. In primarily tuberculous cases the organism adds nothing important to the clinical symptoms. When the infection is primary the symptoms are those of chronic tuberculosis. A bronchial form which runs the clinical course of a chronic bronchitis has been described.

Pulmonary actinomycosis is comparatively rare, but the microscopic find-

ings are characteristic. In the early stages the diagnosis can be made only by discovering the organism in the sputum. In the later stages it is difficult to distinguish it from tuberculosis, carcinoma, bronchiectasis, abscess, etc., without the characteristic microscopic findings.

Since the last great epidemic of influenza many cases of chronic bronchial and lung diseases have appeared that are often difficult to diagnosticate without careful bacteriologic examination of the sputum. Many such cases resemble tuberculous disease, but because of the consistent absence of tubercle bacilli they have been called "localized bronchitis," "subacute and chronic nontuberculous lung infection," "a lobular form of bronchopneumonia of long duration," etc. Field in discussing such cases says that the onset may occur at any age, but is commonest in children. The syndrome usually follows acute disease, which particularly affects the upper respiratory tract, such as measles, whooping-cough, and influenza. In some cases it comes on insidiously. Both the onset and exacerbations occur most frequently during the colder months, probably owing to crowding in ill-ventilated rooms. Chronic sinusitis is probably present in all the cases. *B. influenza* is the most frequent predominating organism, but any of the common invaders, streptococci, staphylococci, pneumococci, and micrococcus catarrhalis, may appear. The process tends to pulmonary fibrosis, or, later, to bronchiectasis and interstitial pneumonia. In these cases the x-ray is of assistance only in locating the lesions, which are usually not typical as in tuberculosis.

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—P. G. W.



# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, MARCH, 1922

No. 6

## *ORIGINAL ARTICLES*

### AN EXPERIMENTAL INVESTIGATION OF THE PHARMACOLOGICAL ACTION OF QUINIDINE\*

BY D. E. JACKSON, PH.D., M.D., ALFRED FRIEDLANDER, M.D., AND  
J. V. LAWRENCE, B.S.

WE HAVE recently heard a well-known pharmacologist make the suggestion that a now very extensively used medicinal compound which has been in the hands of the medical profession for only a few years was introduced into therapeutics at a time when its general toxic action, the size of its fatal dose and its specific action on the various vital organs and functions of the body were almost entirely unknown. These things have since been very largely determined—mainly by trial on the human species. And the same investigator has further suggested that in the alkaloid quinidine we have a drug whose general toxicology, and whose specific actions on the heart, respiratory center and other vital organs should be determined, in so far as may be possible, by animal experimentation before the necessity is at hand of accumulating data on such points as these by the results of disastrous experiments on the human subject.

Quinidine is the dextrorotary isomer of quinine. The melting point of quinidine is 168-171.5°. It is soluble in ether and alcohol, less so in chloroform and benzol and but slightly soluble in water. The sulphate is the usual commercial salt and the common impurities consist generally of traces of hydroquinine and hydroquinidine. If 0.2 gram of quinidine be added to 2 c.c. of concentrated sulphuric acid and then 0.5 c.c. of hydrogen peroxide be added, a yellow color will be produced. This gradually turns to deep orange and finally fades out entirely.

In the present work we have used different samples of the sulphate as prepared by Powers, Weightman and Rosengarten, and also a sample of the

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pure alkaloid as obtained fresh from C. A. F. Kahlbaum. The latter preparation was dissolved by the addition of a small amount of acid. All samples have possessed identical actions on the body so far as we were able to determine.

The recently discovered action of quinidine on auricular fibrillation and its promise of extensive clinical utilization is a therapeutic finding of the first importance. Its miraculous effects in some cases and its apparently fatal action in a certain number of other cases renders a thorough, comprehen-

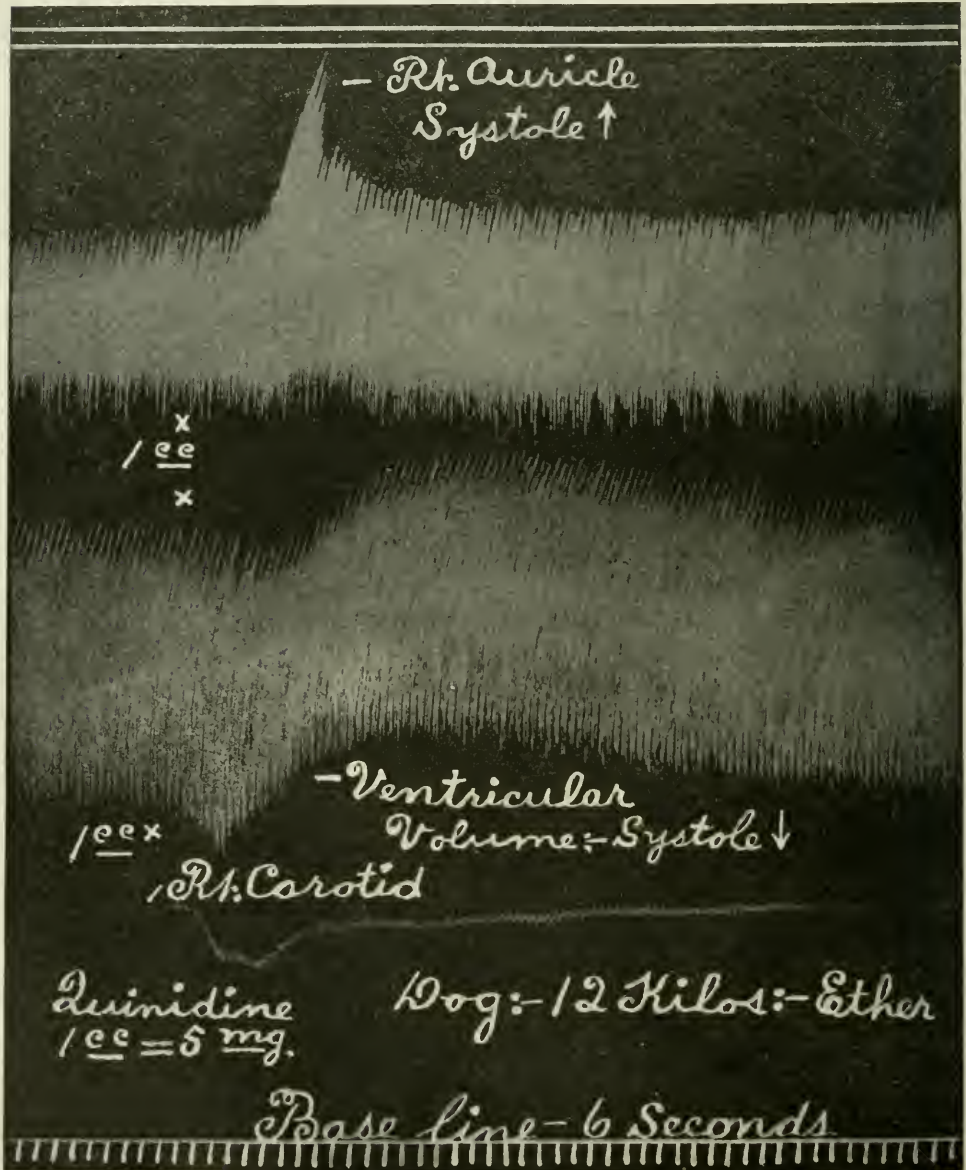


Fig. 1.

sive and accurate study of its actions in the body exceedingly desirable. This will of necessity require a long time. From the clinical papers appearing to date, it would seem that no key to the determination of what cases should, or should not, be exposed to quinidine treatment has yet been found. The best suggestion has simply been the advice to employ the drug with caution, beginning with one or two small doses (0.2 gram) to test the patient for idiosyncrasies, to use the drug only in well ordered hospitals and to keep the patient under constant, careful observation preferably by means of the string galvanometer or polygraph, records being made at short intervals while the drug is being administered. Notwithstanding all precautions it seems that a few unfortunate, and even fatal results, have been brought about, in a few instances after a normal sinus rhythm had been established, and the patient had appeared to be making entirely satisfactory progress. Such cases render

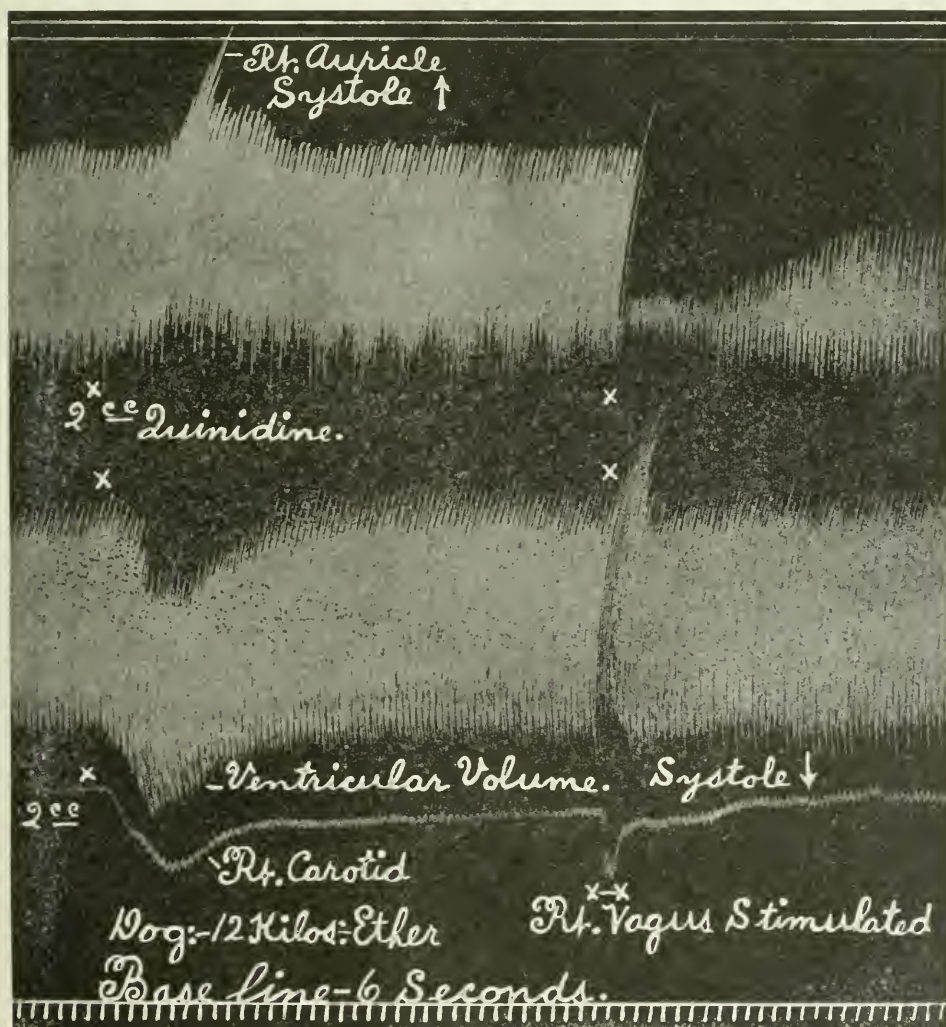


Fig. 2.



a more complete knowledge of the action of the drug, and the indications for its use imperative.

In the present work we have primarily studied the action of quinidine on the normal animal, but we have varied the conditions and the nature of the experiments over an extensive field for the purpose of gaining information regarding the action of the drug from as many different sources and angles as possible.

We can perhaps best describe our observations by reference to the tracings we have selected for inclusion in this article. Fig. 1 was made from a dog with the chest open. A system of levers connected by means of a thread passing over two pulleys was used to record the action of the right auricle, while the ventricular volume was recorded by means of an ordinary cardi-

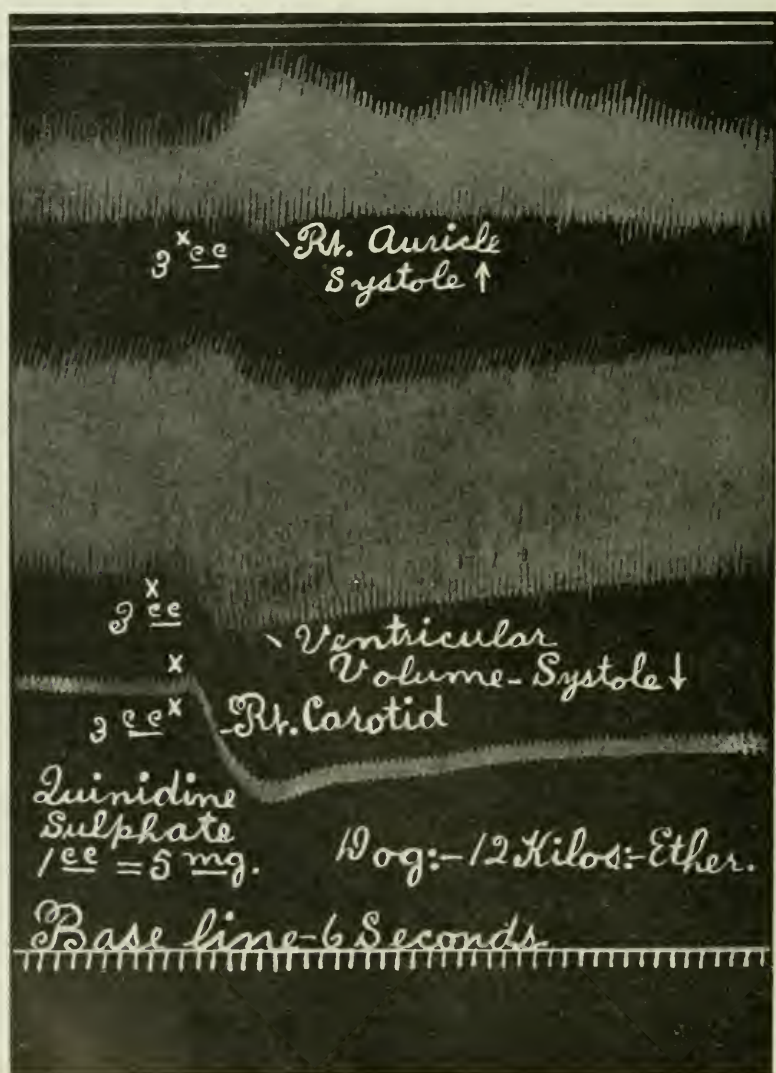


Fig. 3.



ometer connected to a recording tambour. Blood pressure was taken with a mercury manometer. At the point indicated by the cross, 1 c.c. (5 mg.) of quinidine sulphate solution was injected by way of the femoral vein. Contrary to what might be expected, the auricular rate was accelerated and the extent of the systole appeared to be somewhat increased. The ventricle showed a dilatation, but the extent of diastole and systole were but slightly affected, if indeed they were changed at all. The blood pressure fell, and this is the most characteristic and typical action of the drug when injected intravenously.

Fig. 2 (which was made from the same animal following Fig. 1) shows the result of injecting 2 c.c. (10 mg.) of quinidine. Again the auricle is apparently stimulated while the blood pressure falls. The shrinkage in total volume of the ventricle at first is probably due to the general fall in systemic arterial pressure, rather than to a direct action on the heart. As the pressure slowly rises, the heart volume comes back to normal and perhaps a little beyond; i.e., a slight further ventricular dilatation occurs. Thus far the animal had received 15 mg. of the drug. The right vagus nerve was then isolated in the neck and stimulated. At once a marked inhibition of the heart was produced. We suspect the inhibition of the auricle was a little more marked, and a little more prolonged than would have occurred if the quinidine had not been given.

Fig. 3 was made still later in the experiment. At this time the auricular beat was feeble, but the blood pressure remained fairly high. An injection of 3 c.c. (15 mg.) was given and a marked increase in both rate and amplitude of the auricular contractions was seen at once. In fact, the auricular tracing bears a striking resemblance to one produced in a weak heart by a small dose of epinephrin. From most published work one would not expect to find such action on the auricle. The systemic pressure is here again lowered by the injection while the volume of the ventricle shrinks. The extent of its filling and emptying, however, is but little, if any, affected.

Fig. 4 is a tracing made from a different animal. In this case, the auricular record was made by a lever as in Fig. 1; but the ventricular record in Fig. 4 was made by means of a myocardiograph, which records primarily only the systole and diastole of the ventricle. Here it is seen that an injection of 2 c.c. (10 mg.) again stimulates the auricle. And even the ventricular tracing indicates stimulation rather than depression. The typical fall in blood pressure is again produced. It is probable that part of the alteration in rate and amplitude of the heart chambers, as indicated by the tracings, is due secondarily to the fall in blood pressure. This would tend to allow the heart to beat faster, and the increased speed would cause the very light writing levers to manifest a certain amount of fling as a result of the momentum which they acquire in their upward and downward movements. But this cannot account for all of the change which the drug produces in the heart chambers.

Further evidence on this point was accordingly sought by giving quinidine to an animal which had previously received a certain amount of aconitine. This drug possesses a striking action on the heart, and Fig. 5 shows the

peculiar weakening and irregularity produced in the auricle by the drug. The ventricular record was made by means of a myocardiograph. After the auricular record had become very weak and irregular, a dose of 2 c.c. (10 mg.) of quinidine was injected. A prompt increase in amplitude and regularity was produced in the auricle. The ventricular changes were less marked. But in this case the heart muscle must have been in a very peculiar condition as a result of the aconitine action. This drug renders the heart muscle more irritable and with large doses produces great irregularity and arrhythmicity. In the earlier stages the heart is slowed because of a strong medullary stimulation of the cardioinhibitory center by the drug. It has been found that after section of the vagi, or after atropine, a much larger dose of aconitine is required to produce irregularity of the heart than is necessary in

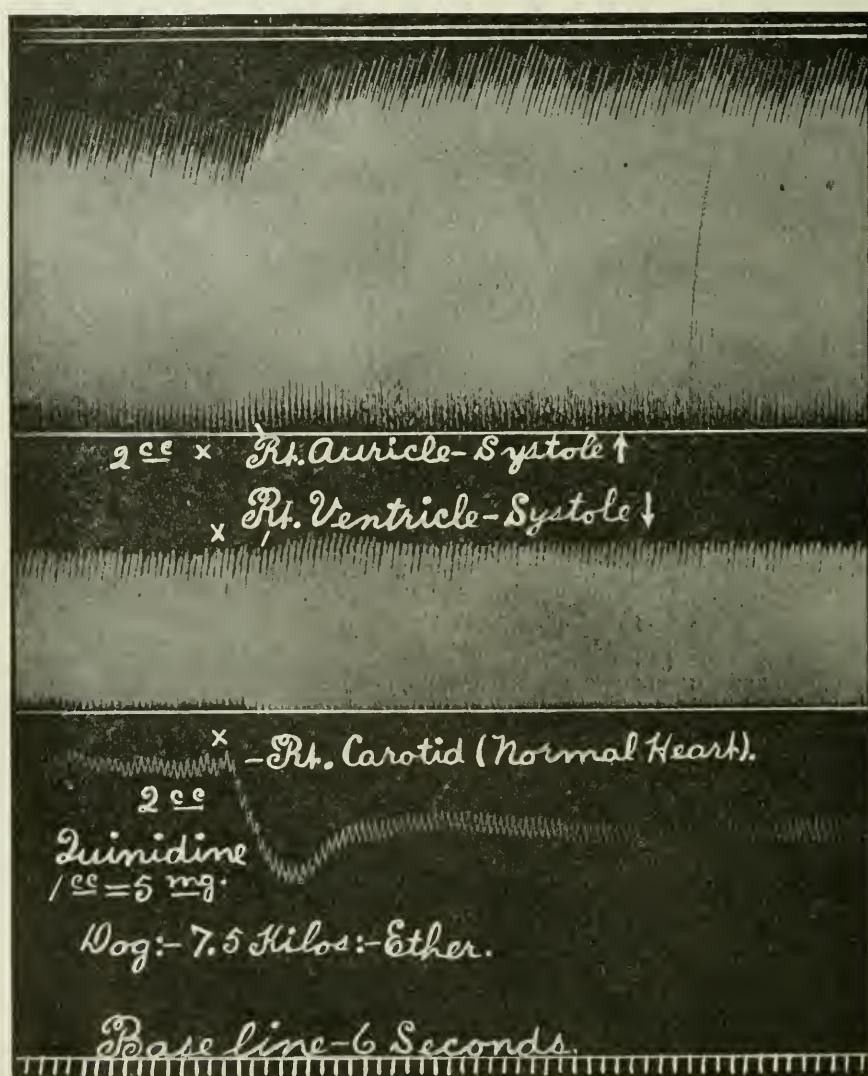


Fig. 4.

a normal animal. We strongly suspect that quinidine in some way must affect the same mechanism in the heart which is involved in this last peculiar action of aconitine. In Fig. 5 it is seen that quinidine actively counteracts the irregularity which had been set up by the aconitine. It is conceivable that if, in this animal, that influence which section of the vagi interposes to hinder or delay development of cardiac arrhythmia had been suddenly increased just at the time the quinidine was injected, then that influence might have restored the heart again to a regular rhythm. In Fig. 6 the action of quini-

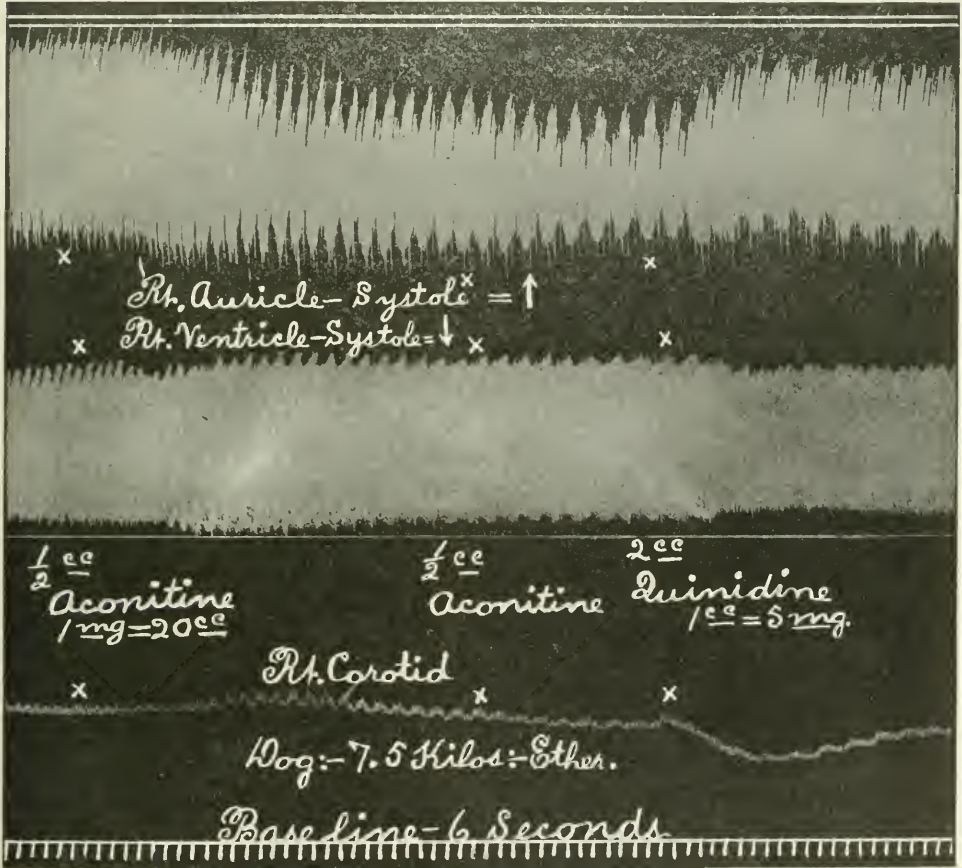


Fig. 5.

dine on auricular irregularity is again shown in a striking manner. The blood pressure tracing here, however, shows that the effectiveness of the heart is not much, if any, improved by the action of quinidine on the auricle. But the stage of aconitine intoxication in which the animal happens to be at the time the quinidine is given has a great deal to do with the results obtained. We have investigated this point by injecting quinidine very early in the stage of aconitine poisoning, when the heart had just developed a marked (and probably lasting) irregularity. Then in other animals the aconitine action has been developed further and further before the initial dose of



quinidine has been given. The following tracings will illustrate clearly the opposing effects which aconitine and quinidine have on the heart.

Fig. 7 shows the earliest development of cardiac irregularity just after a dose of  $\frac{1}{4}$  c.c. of the aconitine solution had been injected. More aconitine had been injected previously in very small, repeated doses as it was desired to bring on cardiac irregularities very gradually. Some central vagus stimulation was present here as indicated by the gradual fall in pressure in the beginning of the tracing. The blood pressure (mercury manometer) shows fairly well the action going on in the heart. The irregularity which suddenly appears after the aconitine is injected, therefore, is influenced by both the central inhibitory action and the direct muscular stimulation of the aconitine. It is probable that an irregularity of this particular type would not cease abruptly and automatically without some special influence being brought to bear on the heart. Consequently it is fair to presume that the injection of 1 c.c. (5 mg.) of quinidine caused the heart to return to an approximately

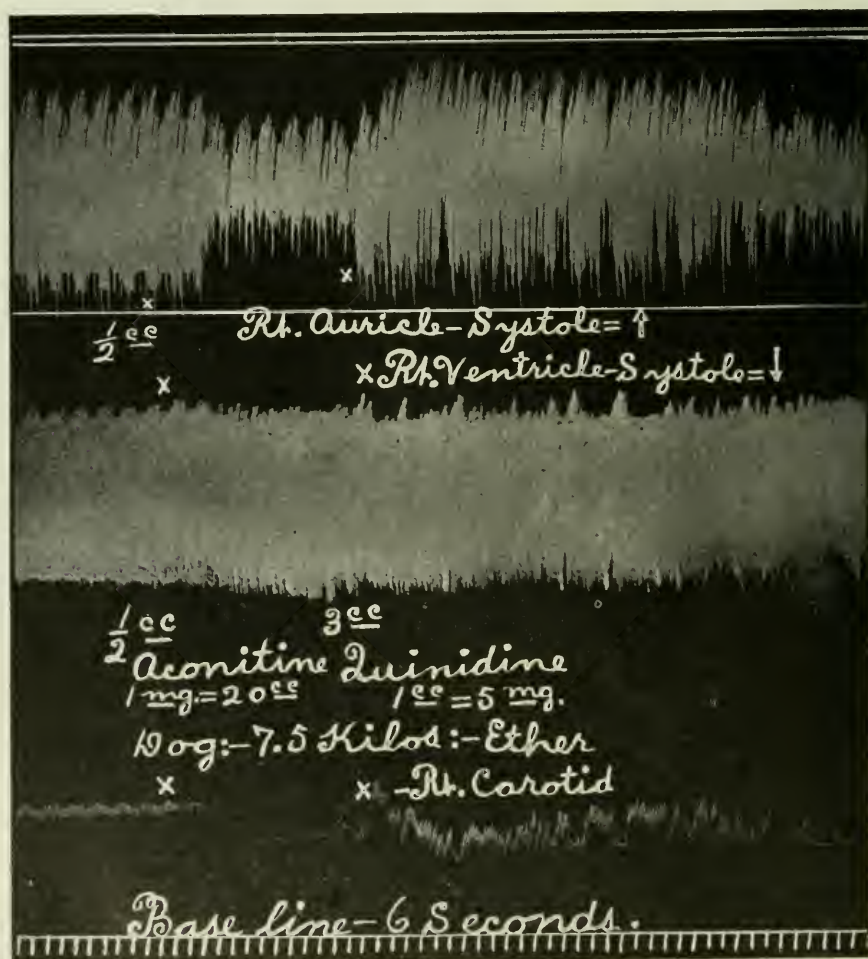


Fig. 6.



normal rhythm. After the initial fall the pressure again rose to approximately its normal level and the respiration again improved, probably partly because of the improved circulation. It can be noted, however, that after a time the effects of the quinidine tend to wear off and the aconite action reappears

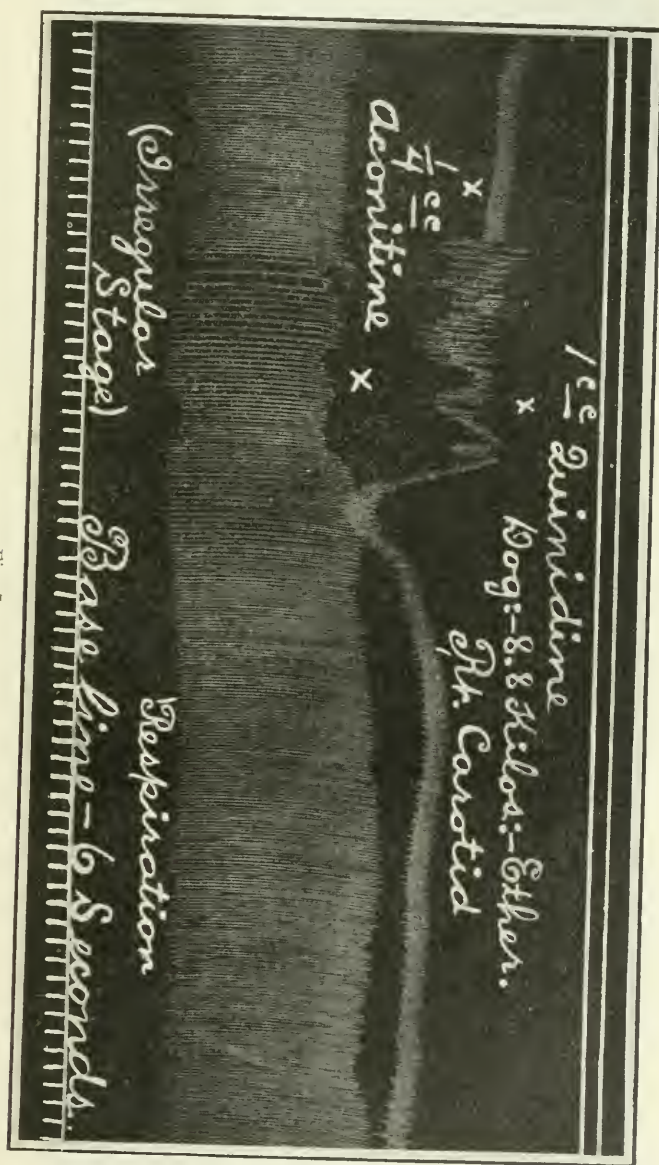


Fig. 7.

even though no further injections of the drug are made after the quinidine is given. This temporary character of the quinidine action is perhaps similar to that observed over periods of a few hours, or a few days in clinical cases in which fibrillation of the auricles is checked temporarily, but in which a recurrence of the fibrillation soon occurs.

Fig. 8 shows a more marked development of aconitine irregularity before

the quinidine is injected. In this case, following  $\frac{1}{10}$  c.c. of aconitine solution, there is developed a very marked central vagus stimulation as shown by the fall in pressure and the long, slow heart beats. The cardiac muscle is also

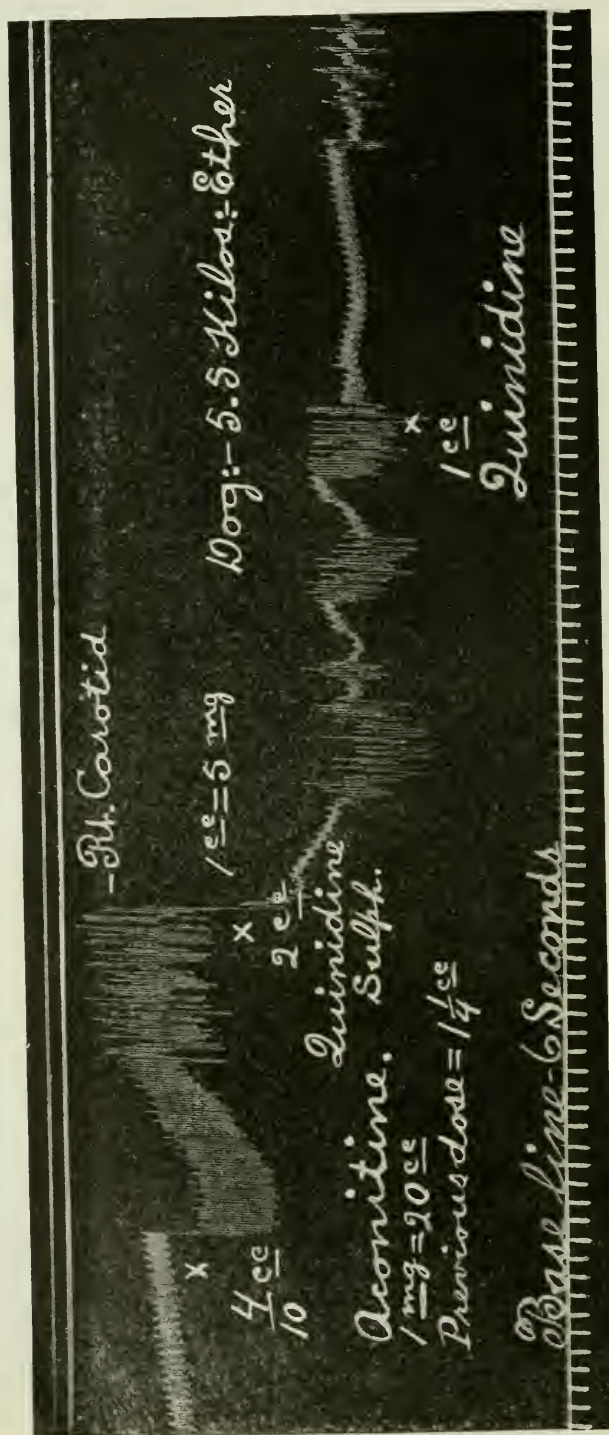


Fig. 8.

becoming highly irritable as is shown a little later by the sudden irregular appearance of extrasystoles, missed ventricular beats, and perhaps other types of arrhythmia. It is certain that the heart here would not spontaneously have returned to a normal rhythm. Therefore, 2 c.c. (10 mg.) of quinidine

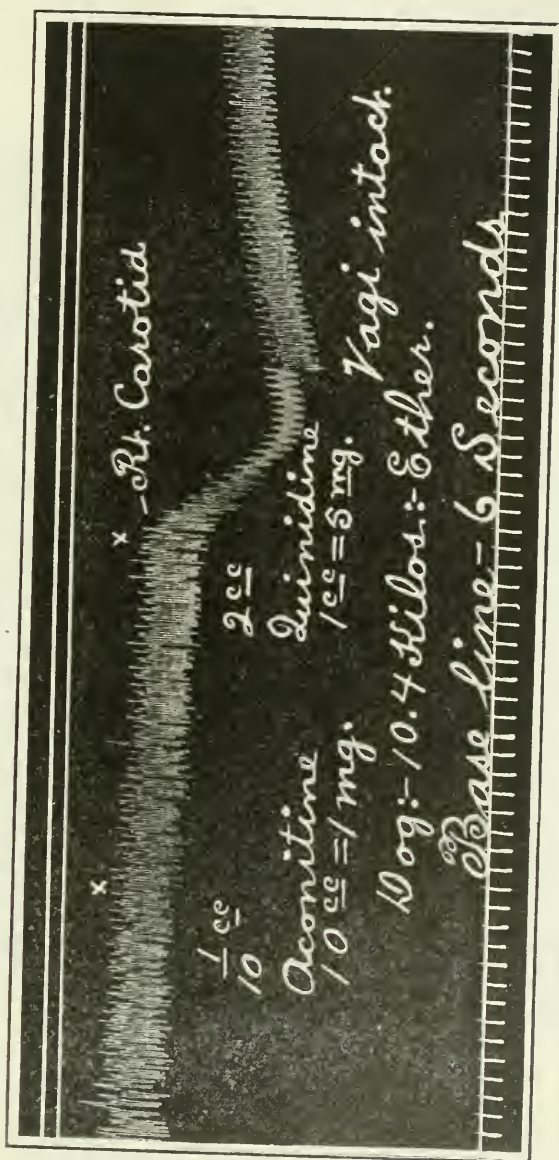


Fig. 9.

were injected at the point indicated. A prompt fall in pressure followed, but for a short time the heart was restored to a normal rhythm. The aconite, however, continued to act and in a short time the previous arrhythmia again started up. This continued intermittently for a time, and at the point indicated a second injection (1 c.c., 5 mg.) of quinidine was given. This time a completely normal sinus rhythm was produced and continued for more than



a minute, after which the aconite action again began to manifest itself. This tracing shows quite well the counteracting action which quinidine possesses against poisonous doses of aconite, if the quinidine be injected fairly early in the course of the intoxication.

Fig. 9 shows this same point, but here the action of the aconite (repeated doses) had progressed still farther before the quinidine was given than was the case in Fig. 8. The strong vagus inhibition is shown in the latter part

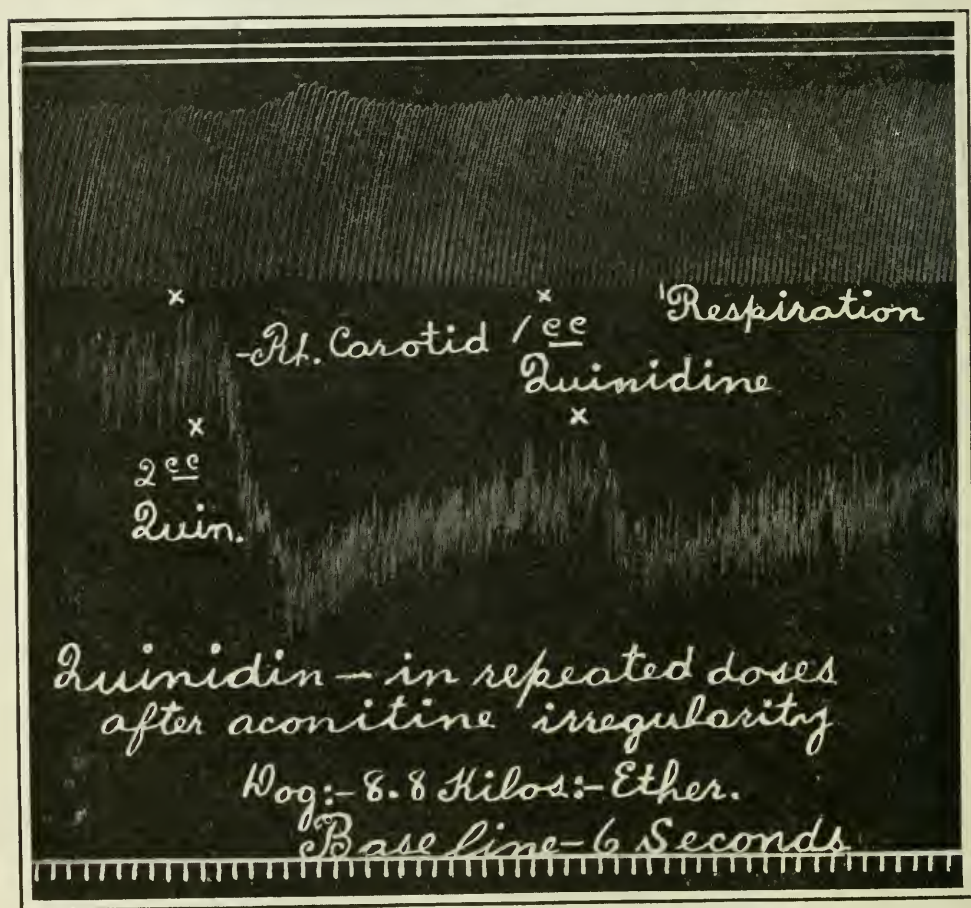


Fig. 10.

of the tracing as it slowed the heart and checked the regular sinus rhythm which the quinidine had temporarily established.

Fig. 10 (which was made from the same dog as that used to produce Fig. 7) shows the complete failure of quinidine to overcome the cardiac irregularity which is established in the late stages of aconitine poisoning. This point is of some clinical interest because of the failure of quinidine to stop auricular fibrillation in a certain number of cases. These cases cannot now be known beforehand because there are no known signs or symptoms to serve as a basis for selection. But the fact that quinidine will stop auricular



fibrillation in about 50 per cent of all clinical cases, while in the remaining 50 per cent some cases can be changed from a fibrillation to a flutter, others show no change, while in still others an acceleration may apparently occur,

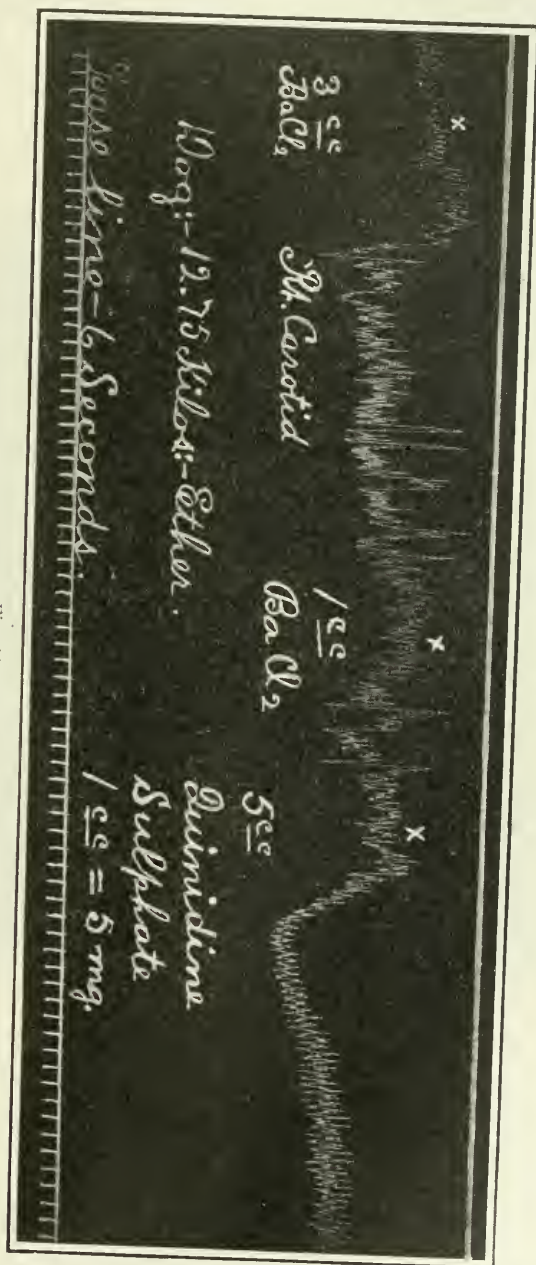


Fig. 11.

indicates that there are probably at least two types of auricular fibrillation. It is to be noted, of course, that aconite sets up irregularities in both auricles and ventricles and that these irregularities may not be analogous to those occurring clinically as auricular fibrillation or auricular flutter. On the other

hand, we have selected aconite, digitoxin and barium to set up cardiac arrhythmias because these drugs come more nearly to producing such cardiac irregularities as probably occur clinically than any other drugs with which we are acquainted. And furthermore these drugs involve both the nervous mechanisms controlling the heart, and the heart muscle itself in their actions. Consequently, it was hoped that a study of the action of quinidine on the irregularities set up by these drugs might throw some light on the action of quinidine itself.

Fig. 11 shows the development of a marked (muscular) irregularity in

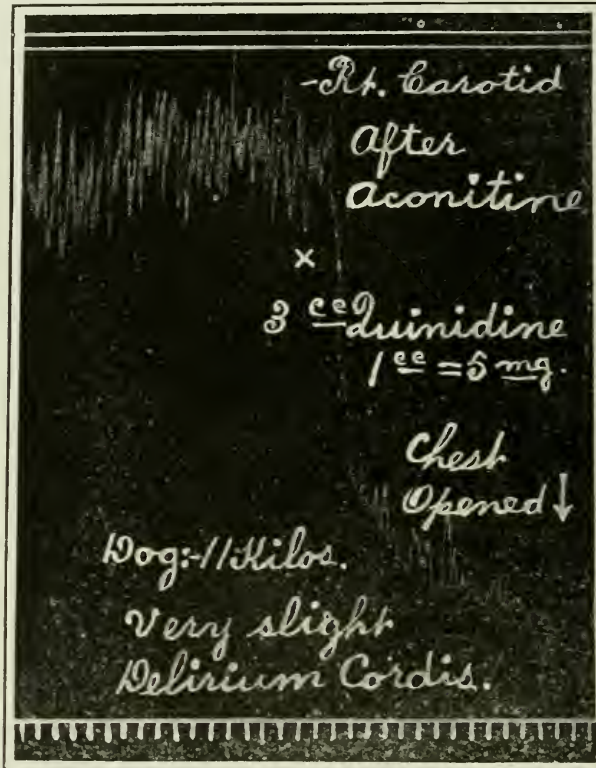


Fig. 12.

the heart as the result of repeated injections of barium chloride. The tracing was made with a mercury manometer. Presumably these irregularities are of purely muscular origin. It will be seen that an injection of 5 c.c. (25 mg.) of quinidine produced a distinct regulatory effect on the cardiac mechanism. While this is less marked, perhaps, than is that following quinidine in the early stages of aconite irregularity, it is still fairly evident and is of interest because here quinidine is counteracting a presumably purely muscular type of irregularity. In the later stages of barium irregularity quinidine is also unable to restore a normal rhythm. Clinically it has been observed that cases of auricular fibrillation of long standing appear to be much less amenable to quinidine treatment than are recent cases.

Cases in which death occurred after quinidine, apparently rather suddenly in some instances, have prompted us to include here Figs. 12 and 13. In Fig. 12 the animal had been given aconitine until a marked irregularity of the heart appeared. The pressure was high, and the possibility, of course, existed that delirium cordis might occur at any time. We then injected 3 c.c. (15 mg.) of quinidine solution. Almost at once the heart was stopped, presumably by the quinidine. We hurriedly opened the chest and found only a very slight delirium cordis to be present. We have repeatedly seen this counteracting action of quinidine, which either partially or entirely prevents the development of a delirium cordis after such drugs as aconite or digitoxin. Fig. 13 shows an abrupt fatal termination caused by an injection of quinidine following marked cardiac irregularity (3rd stage) produced by digitoxin.

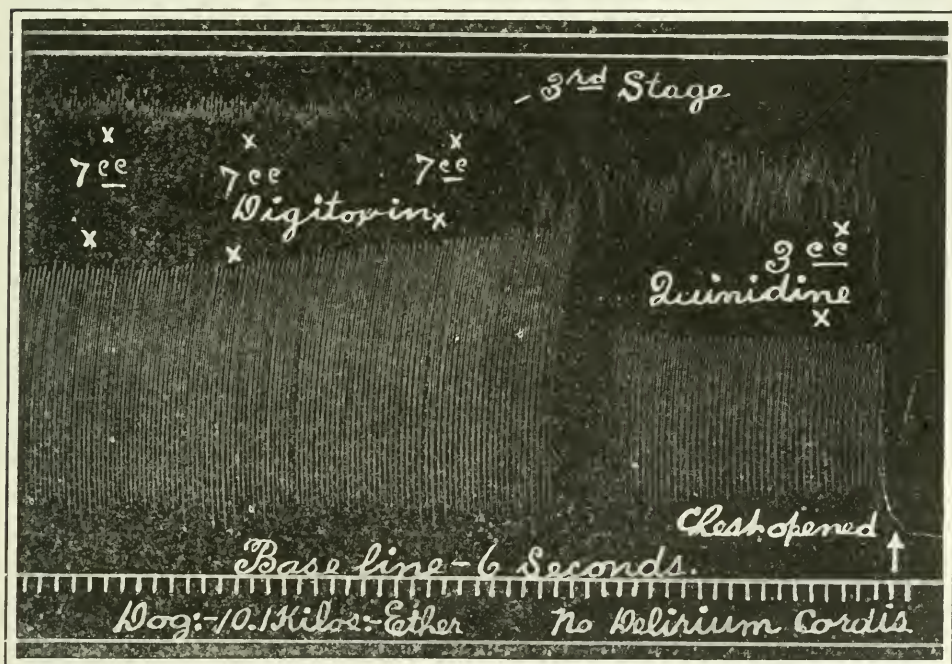


Fig. 13.

The chest was opened immediately and no delirium cordis was found as would have been expected from the action of the digitoxin.

Figs. 14 and 15 represent another type of experiment in which cardiac irregularities were established by a different method. In this case the chest was opened and the heart exposed. With a fine pointed hypodermic syringe an injection of a few minims of a mixture consisting of equal parts of alcohol and chloroform was made into the base of the ventricle at the position of the auriculoventricular junction. The object was to establish heart block by injury to the bundle of His. In this type of experiment it is possible to produce auricular fibrillation or flutter by direct Faradization of the auricles. As will be seen from the tracings marked irregularity of the heart was



produced. We assume that this irregularity may bear some resemblance to that occurring clinically in auricular fibrillation or flutter. In both tracings it can be seen that quinidine exercised a distinct regulatory effect on the heart beat. And this effect appears to be rather more lasting in its character than is that which usually follows irregularities produced by aconite, digitoxin or barium.

Fig. 16 is presented for the purpose of demonstrating one point. It has



Fig. 14.

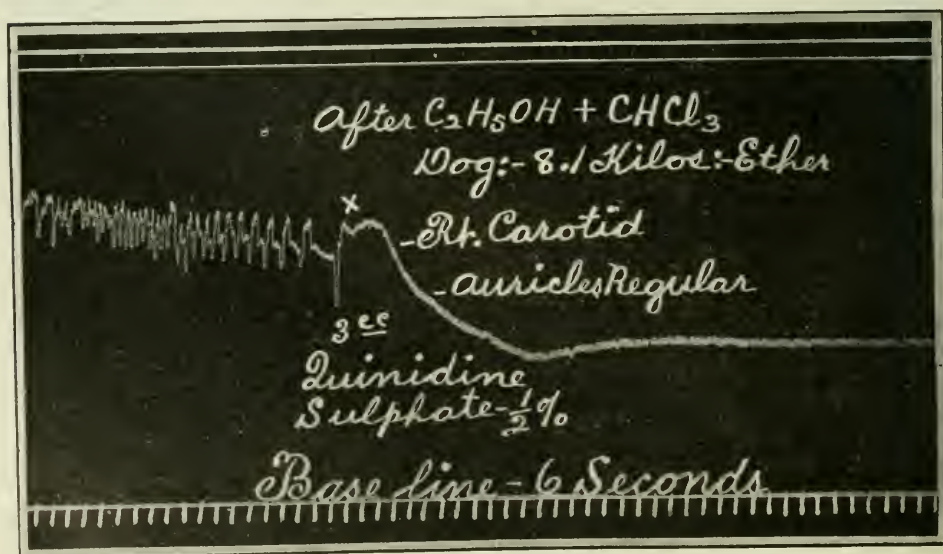


Fig. 15.



been supposed that quinidine paralyzes the inhibitory endings of the vagi in the heart. This action has been described as being similar to, but milder than, that of atropine. In Fig. 16 the records of the auricular and ventricular contractions are shown. The animal had previously been given 100 milligrams of quinidine in small repeated doses. At the time when this tracing was made the blood pressure had been reduced to a very low level by the quinidine and we feared to give another dose of the drug because it would probably stop the heart (the animal was getting artificial respiration). Our object was to give the quinidine all possible opportunity to act on the vagi

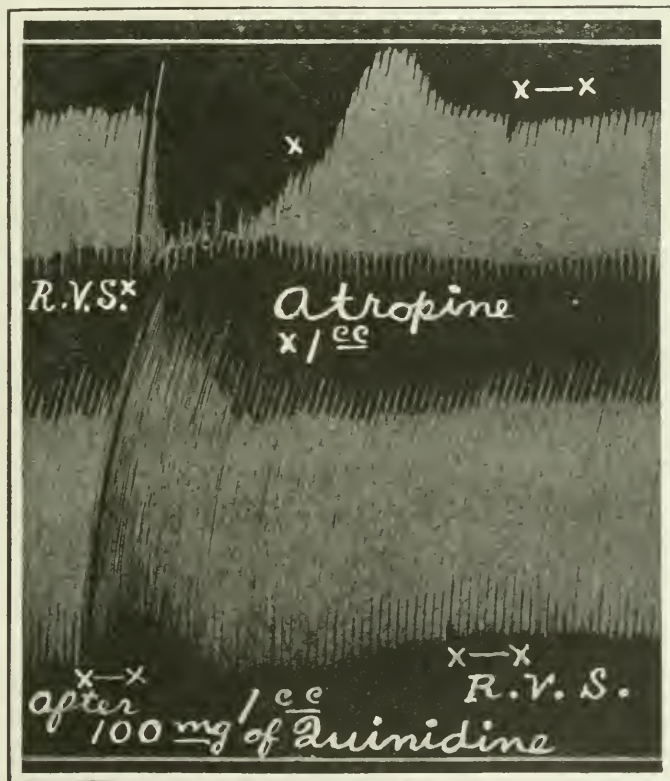


Fig. 16.

endings and then to determine by electrical stimulation of the nerve in the cervical region whether inhibition of the heart could still be produced. At the point indicated in the left of the tracing the right vagus nerve was stimulated and a prompt complete inhibition of the heart was produced. This lasted longer in the auricle than in the ventricle, which presently broke through the inhibition, but still showed its effects while the stimulation was continued. Following this, and while the inhibitory effect was still well marked in the auricle, an injection of 1 milligram of atropine was given by way of the femoral vein and this promptly stopped the lingering inhibitory effects in the auricle which responded by a marked increase in both rate and amplitude of its beat. Following this the right vagus nerve was again stim-

ulated, but no inhibitory effects were produced on the heart. We conclude from this (and other similar experiments) that not only are the vagus endings not paralyzed by quinidine, but that the inhibitory action of the vagi on the heart is, if anything, actually increased by quinidine. And we suspect that this action is of immediate importance in the matter of the checking of auricular fibrillation or flutter by quinidine.

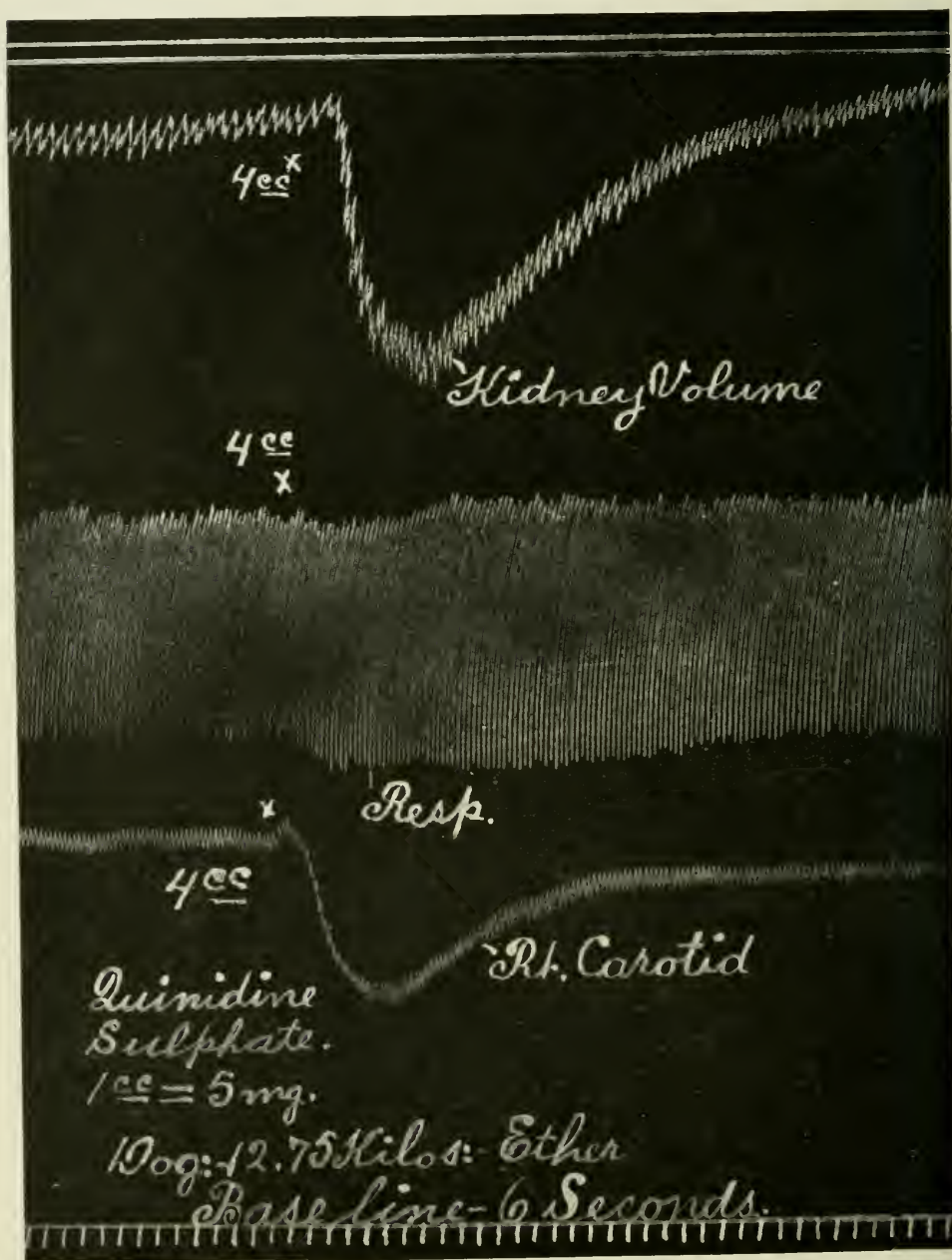


Fig. 17.

This brings us to another phase of the action of quinidine. We have regularly observed that when an intravenous injection of adrenaline is given to a normal animal and a blood pressure tracing is made, then if one or two injections of quinidine be administered and this be again followed by adrenaline, it will be found that the animal is strikingly less sensitive than at first to the action of the adrenaline. Indeed this result is so marked that when we first observed it we were inclined to suspect that quinidine possessed a paralyzing action on the sympathetic nerve endings similar to that of ergotoxine. And we suspect that large doses of quinidine do actually depress the vascular sympathetic nerve endings. Such an action probably also occurs in the case of the sympathetic nerves to the heart. And this may be of considerable importance in lessening the irritability of fibrillating or fluttering auricles in clinical cases. This action on the sympathetics is best seen, however, in the blood pressure. For after large or repeated doses of quinidine, adrenaline may be

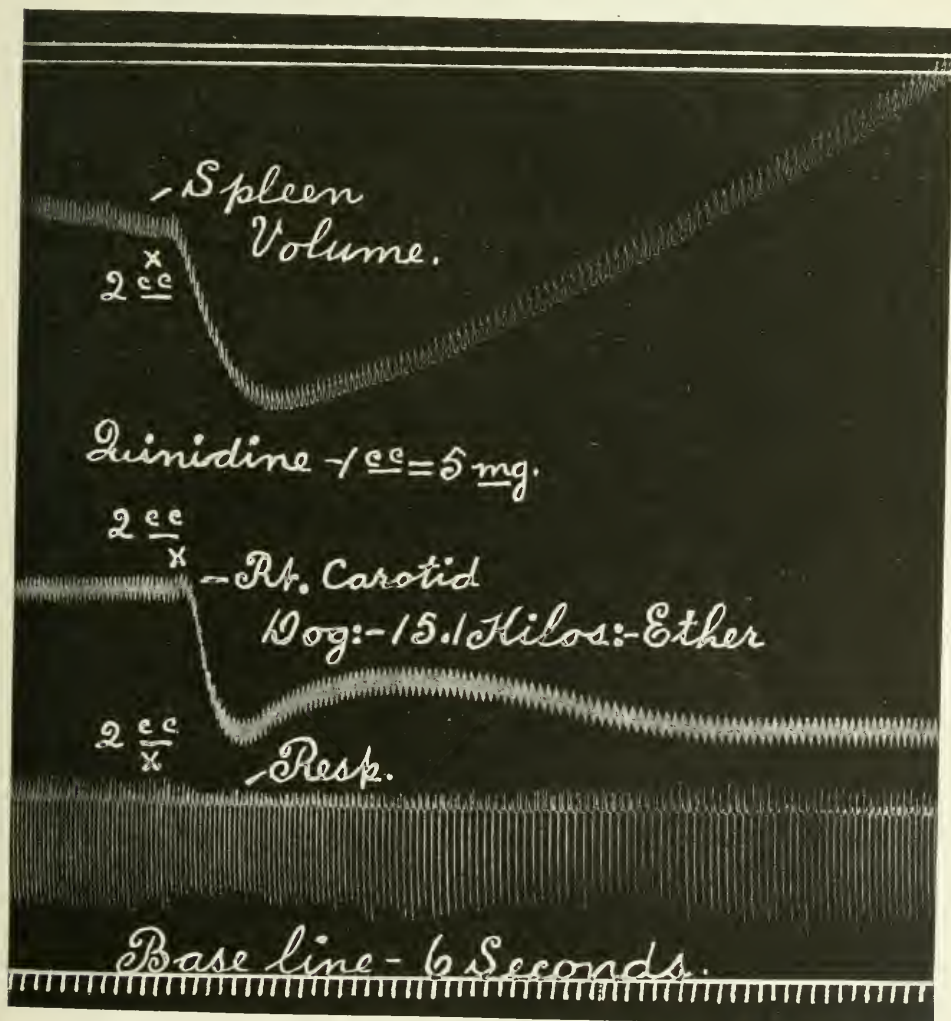


Fig. 18.



found to be very much less effective in producing a rise in pressure than it had been before the quinidine was administered. There are other features to this problem, however, and we may defer its further consideration until we discuss the peripheral action of quinidine.

Figs. 17 and 18 show the action of quinidine on the respiration, blood pressure and the volume of the kidney and spleen when the drug is injected intravenously. It will be noted that both the kidney and the spleen shrink in volume. We suspect that this is due to at least two causes. First, the general fall in blood pressure allows the kidney and spleen to shrink somewhat by their own elasticity. And further, the fall in systemic pressure sets

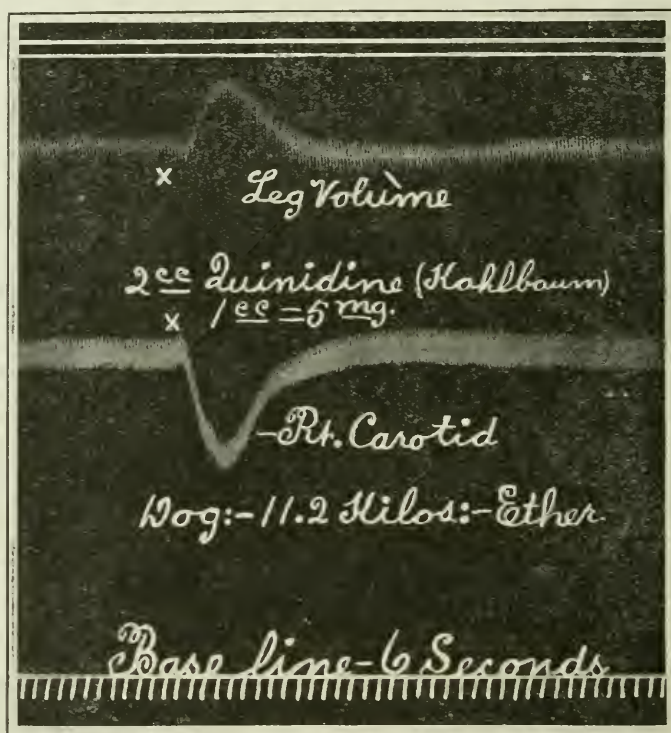


Fig. 19.

up a certain amount of asphyxia in the medullary vasomotor center. This center will respond by contraction of the visceral vessels and this action is probably concerned in the decrease in volume of the kidney and spleen as here shown. We believe (although we have not yet proved) that there is no *direct* action on the kidney or spleen which would tend to cause them to contract.

Figs. 19 and 20 illustrate another point in the general action of the drug. In these records the volume of the hind limb is recorded in the upper tracings. It will be seen that the leg volume increases while the general systemic blood pressure falls when quinidine is injected. This peripheral dilatation we believe probably plays a rather important part in the action of the drug.



We are not sure as yet whether this dilatation of the general systemic vessels as seen in the leg volume represents a central or a peripheral effect, but certain other features of the drug's action lead us to presume that quinidine here acts primarily on the peripheral vessels, and possibly to a considerable

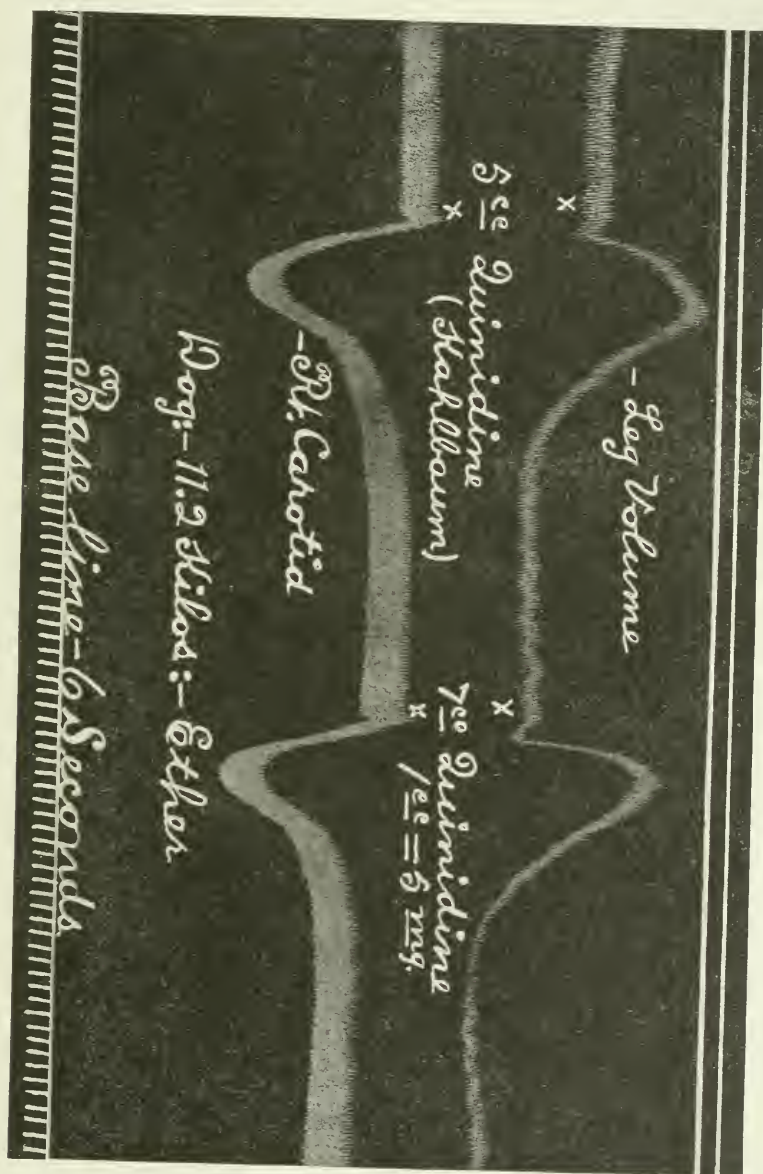


Fig. 20.

extent on the capillaries. We are at present carrying on further investigations along these lines. It was mentioned above that quinidine tends to decrease the sensitivity of an animal to adrenaline. If the drug should do this by slightly depressing the vascular sympathetic endings we would naturally expect to get a dilatation of the peripheral vessels when the drug is injected unless some counteracting influence, such as medullary asphyxia, should be

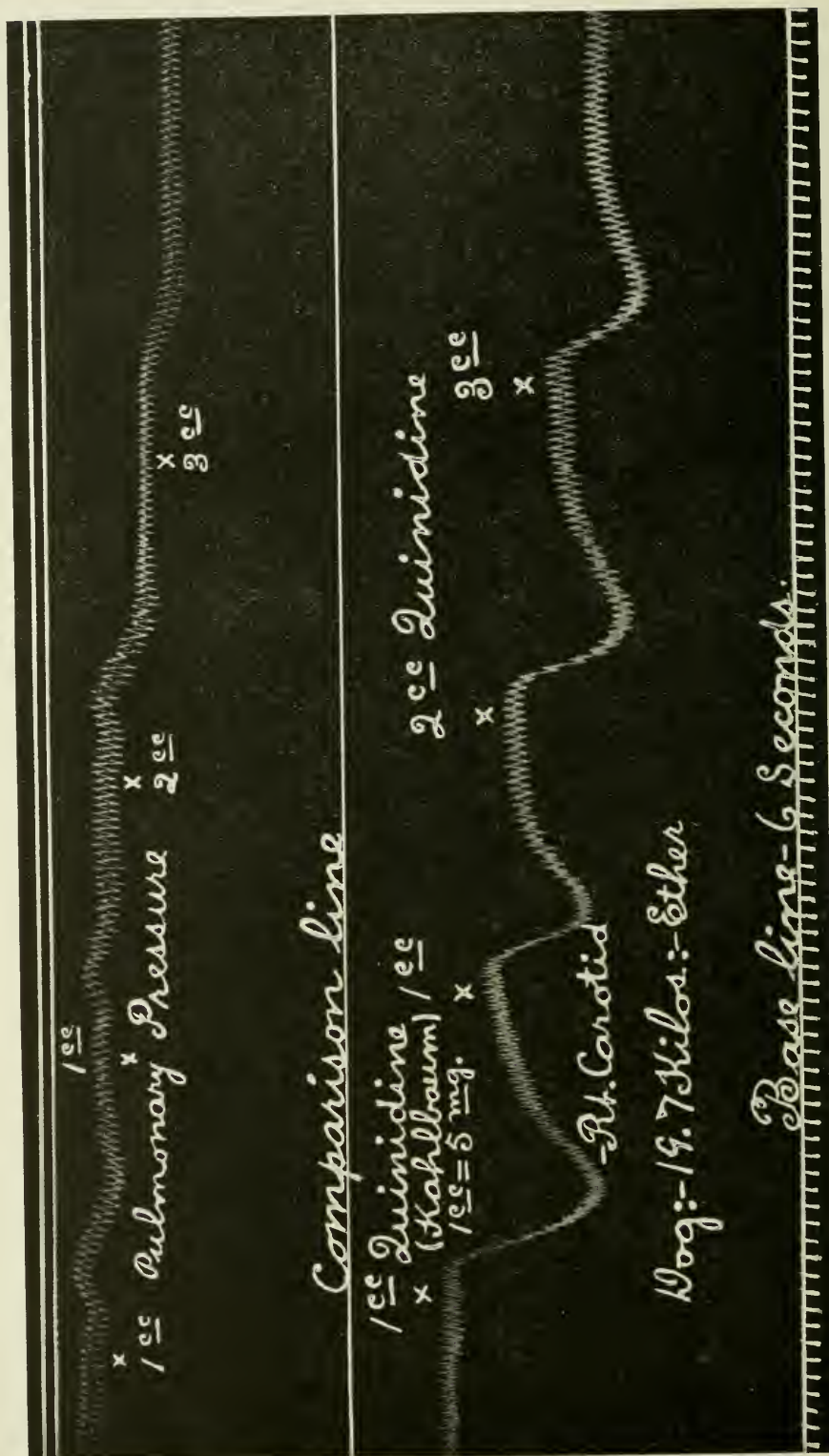


Fig. 21.

brought into play at the same time. It is of interest to mention that a dilatation of the coronary vessels might be of considerable advantage to the heart in many clinical cases. Of course, the muscle fibers of the arteriole walls may be directly depressed by the drug, but it is our impression that the peripheral action of the substance is of greater extent and importance than this. We should like to suggest that a careful clinical study of the general condition of the *peripheral circulation* be made in all patients to whom the drug is given, both before and after the administration. We believe that such investigations

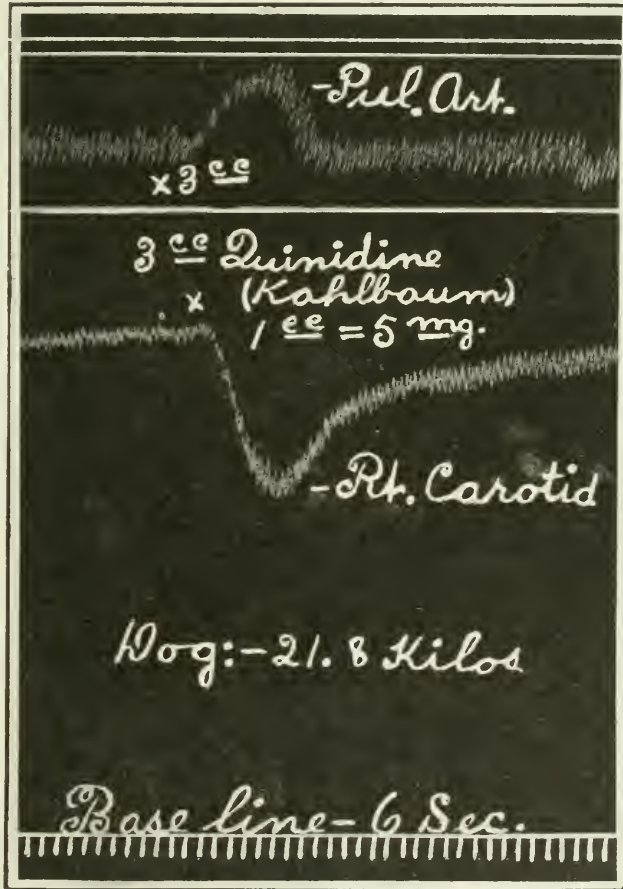


Fig. 22.

may throw considerable light on the question of the proper selection of cases for quinidine treatment.

The pulmonary blood pressure occupies a peculiar relation to the general circulation in cardiac disturbances, such as auricular fibrillation or flutter, and especially so when these conditions are further complicated by valvular disease. Consequently any drug which exercises a considerable action on the pulmonary pressure would be worthy of careful study when used in the treatment of such cardiac disturbances. As will be seen from Figs. 21, 22,



23, 24 and 25, quinidine exercises a marked effect on the lung circulation. Apparently a number of factors are involved in this action. Small doses rather tend primarily to produce a rise in the pulmonary pressure, while large doses always cause a fall, and in many cases this fall is out of proportion to that

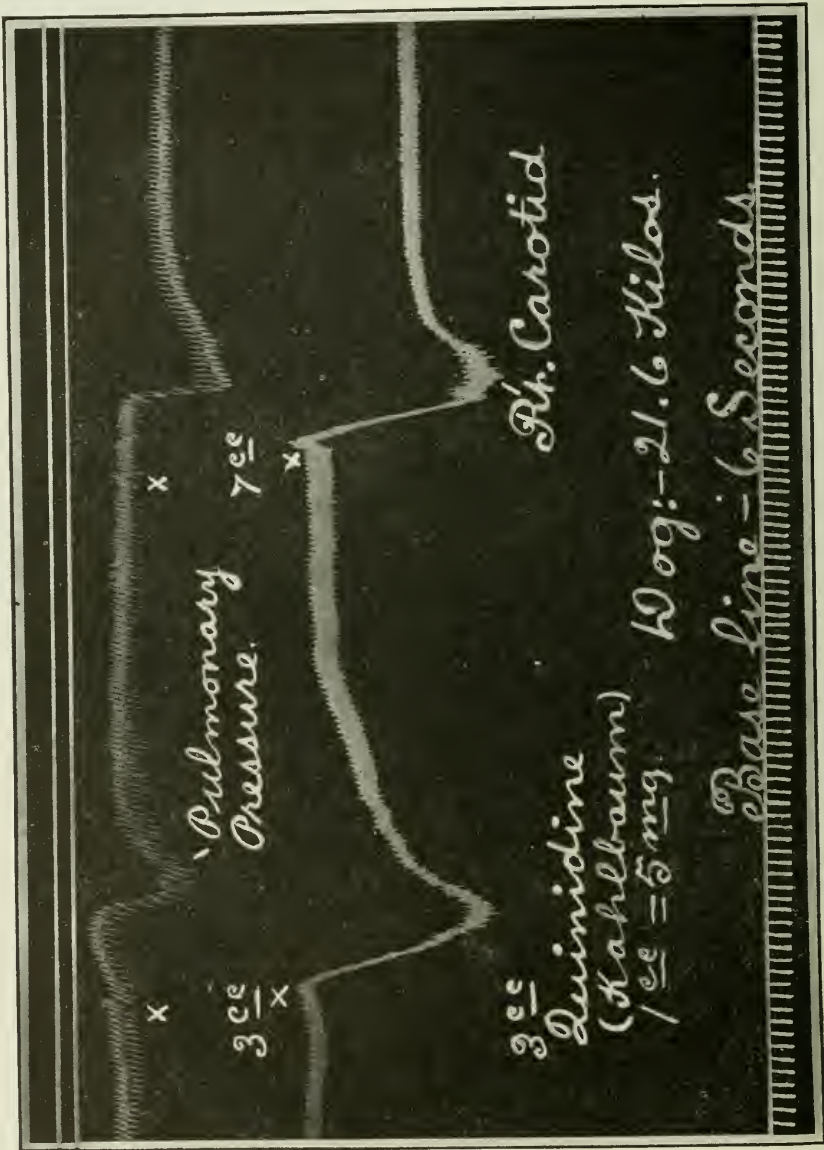


Fig. 23.

which is correspondingly produced in the systemic pressure. We believe that the slight rise produced in the pulmonary vessels by very small quantities is a secondary effect and occurs as a result of the fall in systemic pressure, or by a damming back of the blood in the heart. It is probable also that in some cases the early acceleration, and even stimulation, of the heart such as is shown in Figs. 1 and 2 may so affect the right ventricle that a slight



increase in the volume of blood thrown into the pulmonary arteries may occur for a brief interval. It seems certain, however, that larger doses of quinidine injected directly into the circulation will always produce a fall in pulmonary pressure. Obviously if any such action as this should occur clinically it might have a great deal to do with the outcome of the treatment. We have tried to reduce the conditions of our experiments as nearly as possible to those of the patient who may be treated with quinidine. Of course, the normal tissues and organs of a patient with a cardiac lesion will be affected by the adminis-

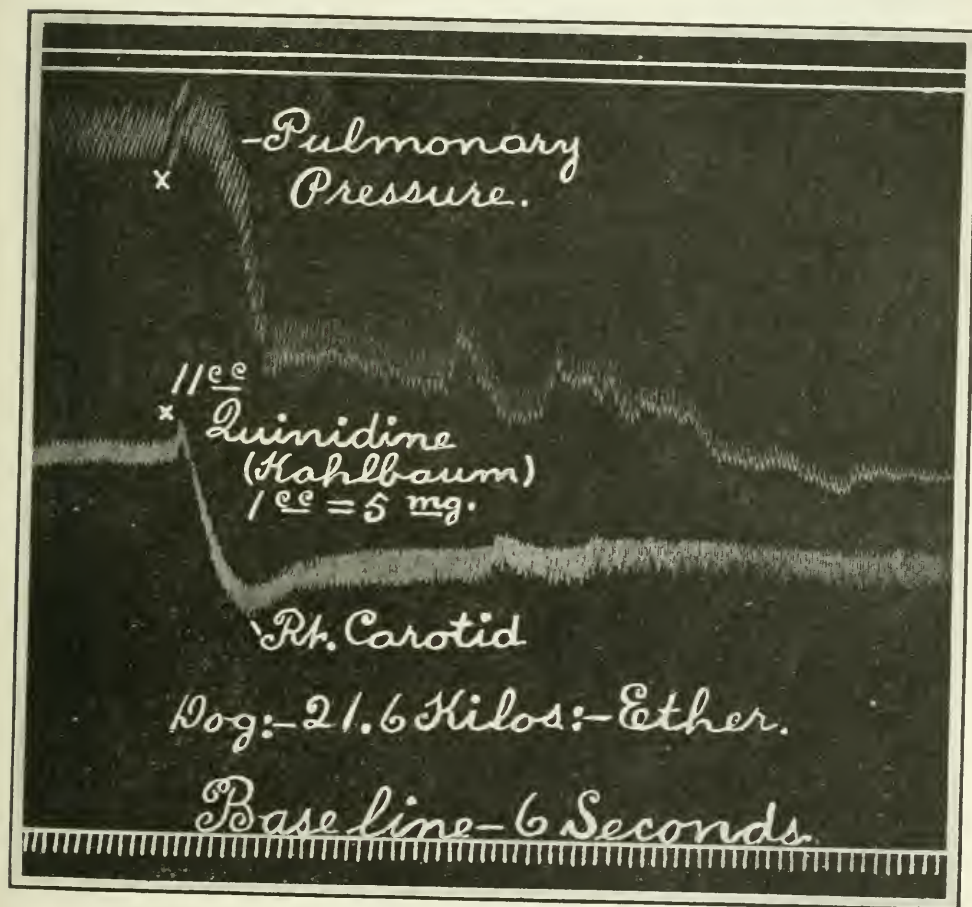


Fig. 24.

tration of quinidine in a manner analogous to that which would occur in a normal individual. And through a gradation of dosage we have here attempted to find out whether it is probable that a fall in pulmonary pressure occurs in the clinical use of the drug. We have recorded the pulmonary pressure by means of a water manometer connected with the left pulmonary artery. The distal limb of the manometer was connected by rubber tubing to a long *inverted* U-tube, filled with air and the distal end of the U-tube was connected with a recording tambour which marked on the drum. This arrangement gave a considerable magnification of the pulmonary pressure

over that of the systemic record which was obtained by means of a mercury manometer.

The proportionate dosage which we have used to obtain pulmonary pressure records has often been below that which is generally used in clinical practice, as indicated by comparing the weight of the animal with that of a patient. Absorption from the stomach or small intestine, of course, requires much more time than does intravenous injection to get the drug into the general circulation. And the regular fall in systemic pressure which the drug produces may have some indirect effect on the lowering of the pulmonary pressure. Judging from other results the depression of the heart muscle, which has generally been supposed to be the chief action of the drug on the circulation, is not sufficient to account for all of the fall in the pulmonary pressure. It seems evident, therefore, that a dilatation of the pulmonary arterioles and capillaries occurs under the action of quinidine. And we believe that this action in all probability occurs in many, if not in all, cases in which the drug is used clinically. Such an action would probably be of benefit in many instances, even if the drug had no effect on the heart at all. On the contrary, in other cases such effects might be of no benefit, or might even be harmful. But it is to be noted that the dilatation of the vessels of the hind limb with a consequent increase in volume, and the dilatation of pulmonary arterioles and capillaries both indicate that the drug possesses a distinct peripheral action on the circulation which should be taken into account in its clinical application.

In conclusion we must refer briefly to a point which has recently been especially emphasized by Lewis<sup>7, 34, 42</sup> and others with reference to the nature of auricular fibrillation and its treatment by quinidine. The circulation of contraction waves in muscle (*Cassiopea*) was studied by Mayer<sup>36</sup> as early as 1908. W. T. Porter<sup>33</sup> in 1894, originated the block hypothesis of fibrillation. But later Garrey<sup>31, 30</sup> suggested that fibrillation of the heart muscle probably consisted essentially in a large number of small contraction waves circulating diffusely and simultaneously in many directions and entirely without co-ordination in the heart muscle. These waves were supposed to be conducted directly from muscle fiber to muscle fiber, and the refractory state of fibers which had just relaxed set up blocks against the waves in many places and thus diverted them promiscuously into other channels from which the refractory state had already disappeared. Mines<sup>35</sup> in particular studied this phase of the problem. These views regarding the essential nature of fibrillation have been generally held for a long while. But recently Lewis<sup>7, 42</sup> and his co-workers have introduced the view that in auricular fibrillation a single contraction wave only exists, and that this "is propagated and revolves perpetually upon a re-entrant path. The circus movement is repeated on the average 450 times a minute, and it is this circulating wave which, in its revolution, alone controls the beating of the auricle." The wave as thus conceived would be influenced by three factors "(a) the length of the path followed, (b) the duration of the refractory period at any given point; and (c) the speed at which the wave is conducted." Lewis<sup>7</sup> has suggested that

quinidine may act on auricular fibrillation by either slowing the rate of conduction of the contraction wave (thus tending to increase the length of the gap between the advancing crest of the wave and the retreating area of refractory tissue over which the wave has just passed and into which the

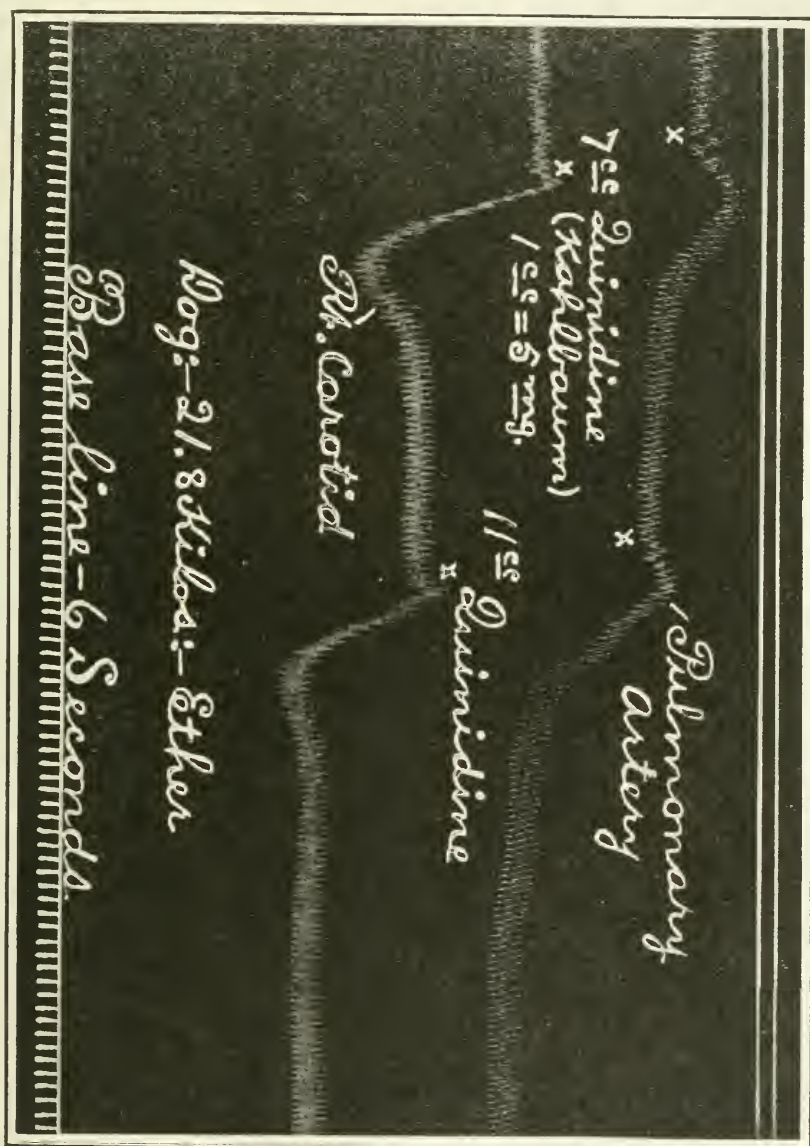


Fig. 25.

contraction wave is progressively reentering), or else by lengthening out the extent of the refractory period in tissue over which the wave has just passed. The first of these actions would tend to prolong the gap between the refractory tissue and the advancing crest of the returning wave and thus favor continuation of the fibrillation. The second action, however, by prolonging the refractory period in tissue which had just relaxed would tend to stop the



fibrillation because the gap between the refractory tissue and the oncoming crest of the wave would be shortened, and if this shortening should become great enough, the crest would meet refractory tissue and the circulating wave would thus be stopped. Presumably the auricle would then be in position to take up its normal beat. Lewis suggests that these two opposing actions may neutralize each other so far as beneficial action on the heart is concerned, or the slowing of the conduction may even make the condition of the beat worse. He also states that (unpublished) experiments have shown that quinidine in doses comparable to those used clinically may lengthen the refractory period by as much as 50 per cent or more.

We are of the opinion that from a pharmacological standpoint this attractive hypothesis is inadequate to explain the whole of the action of quinidine on the heart. And we should not be at all surprised if later work should render it entirely untenable. When we first approached this problem we naturally suspected that there was nothing unique in the action of quinidine on auricular fibrillation, but that perhaps a large number of drugs which exercise a general depressant action on the cardiac muscle would in all probability act in a very similar manner. It appeared to be simply a question of selecting a substance of sufficiently low general toxicity, and one which would be eliminated from the blood but slowly in order that a prolonged, mild depression of the auricular tissue might be produced. The drug undoubtedly acts on both ventricles and auricles. It tends to counteract the development of fibrillation of the ventricle after such drugs as aconitine or digitoxin. And one of the earliest actions on the normal heart consists of a slight dilatation of the ventricle as shown by the cardiometer. The checking of fibrillation of the ventricles<sup>32</sup> in perfused hearts by the temporary addition of small quantities of potassium chloride solution to the perfusion fluid indicates also that quinidine does not possess a unique action in this regard. And it is our surmise that in due course it will be found that quinidine acts on the musculature of the peripheral vessels, and perhaps even on the skeletal muscles, in a manner quite similar to that in which it acts on the heart muscle. Such an action on the coronary vessels would probably be of importance in some, and perhaps in many cases. And the decrease in the power of adrenaline, when injected intravenously, to raise the general blood pressure after quinidine may indicate an action on the sympathetic innervation, although we cannot at present rule out the possibility that this may be due to an action on the capillaries. Slight changes in the pulmonary blood pressure by an action on the pulmonary arterioles or capillaries might also be of importance in many cardiac cases. And finally, as a word of caution, it is interesting to note that Withering introduced digitalis into the Edinburgh Pharmacopoeia in 1783, and in 1785 he published an article on the *Abuse* of the Fox-glove. And the year 1922 finds us still willing to learn more than we at present know about the fox-glove. Hence a word to the wise will be sufficient with reference to the too hasty formation of conclusions regarding the action of quinidine.

The following bibliography, while not entirely complete to date, will be sufficient to enable those who are interested to follow the question still further.



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## BERIBERI: SOME FACTS AND THE POSSIBLE HYPOTHESIS

BY TSANG G. NI, M.S., CHINA

THERE are different names for beriberi and many theories have been offered concerning the etiology of it. "Che-Zee" is the term given to a disease resembling the "wet" form of beriberi in an old Chinese medical book, "Soo-Van" (said to have been written about 2500 B.C.). Since the Don-Dynasty, the term "Char-Chee" has been seen in literature. The Chinese word "char" means foot. The second word "chee" means gas or something which is labile. It has been described in old literatures that there are two varieties of "chee": "inm" (negative or cathodic) and "yarnn" (positive or anodic); and that in the healthy body there is a properly antagonistic, cooperative and harmonized condition among "inm," "yarnn" and circulation. Beriberi was regarded to be due to the unbalanced antagonism of "chee" in the circulation, starting its symptoms in the "char" (foot). In modern times being convinced by the work done in different countries and the cases in the Chekiang Army Hospital, Chinese medical students and instructors have believed that the deficiency in antineuritic vitamine is one factor causing beriberi.<sup>1</sup>

As stated in literatures of different countries, beriberi has been attributed to the lack of harmony between "chee" and circulation to weakened kidneys;<sup>2</sup> to moist weather; to unfit water and soil; to night air;<sup>3</sup> to poisoning by arsenic, by carbon dioxide, by oxalate, by rice, by fish; to a helminth infection; to autointoxication; to nitrogen starvation; to some protozoon infection with a possible insect-carrier; to the phosphorus starvation; to a bacterial infection in the body; to fungi; to a bacterial toxin of an extracorporeal origin. It is not necessary to deal at length with many ancient theories which can be disproved easily.

Among those who have favored the theory that the disease is an infectious one and not due to deficient nutrition may be mentioned: Scheube,<sup>4</sup> Manson,<sup>5</sup> Balz,<sup>6</sup> le Dantec,<sup>7</sup> Jeansalme,<sup>7</sup> Marchoux,<sup>7</sup> Daniels, Wright, Castellani, and Shibayama.<sup>8</sup> Shibayama states that it is not unreasonable to assume that the microorganisms of beriberi are only present in the Orient and, given a predisposing cause, are capable of producing disease. Castellani<sup>9</sup> also holds a somewhat similar view.

To summarize from the evidence, it appears more likely that a parasite will be found to be a spreader of the disease, which makes it more probable that the actual cause will be found to be a protozoon than that it is due to diet.

Marchoux, writing in 1910, believes that a diet of white rice furnishes in the intestine a favorable culture medium for the development of the organism of beriberi, and that the addition of rice bran to the diet renders the condi-

tion in the intestine unfavorable for the development of this organism. There are numerous other investigators who have described various species of protozoa or of bacteria as the cause of beriberi. However, these have not been definitely proved by scientific researches.

In the fall of 1916 there were about two hundred cases of beriberi in the Chekiang Army Hospital. The bacteriologists in the hospital failed to isolate either specific species of bacteria or of protozoa except those well known as the causative agents of other diseases. However, the fact that the causative microorganism for the disease has not been discovered is not a final argument against its infectious nature, for the specific organism in many diseases of an undoubted infectious nature has been sought for as diligently and as unsuccessfully as in the case of beriberi. We have known that there are microorganisms that probably cannot be seen, at least not today, with even the highest powered microscope, and that there are viruses able to pass through the pores of the filters. Shiga and Kusama<sup>10</sup> attempted to obtain the reaction of deflection of the complement in the serum of beriberi patients. The result of their experiments was negative. Grijns and de Haan<sup>11</sup> made experiments to demonstrate the presence of antibodies in the blood of organs of beriberi cases or of fowls suffering with polyneuritis; they were no more successful than we. Riddell, Smith, and Igarevidez stated that cultures made from the heart and lungs at postmortem were negative. No parasites were found in the ileum.<sup>12</sup>

Although we do not want to exclude definitely the possibility of the disease being an infectious one, merely by means of negative experiments, yet, if by scientifically controlled experiments we can produce the disease and exclude the influence of a living specific microorganism, the infectious theory of its origin can be regarded as no longer tenable. Eykman found that fowls develop polyneuritis when fed on polished rice. Grijns,<sup>14</sup> Sakaki,<sup>15</sup> and others repeated and verified the experiments.

Concerning the relation of polyneuritis gallinarum to beriberi there are different opinions. Kusama and Shiga<sup>10</sup> believe that it would be too much to say "The polyneuritis of animals and beriberi are identical." Shaumann<sup>16</sup> states "Not that both diseases are identical but there seem to exist reasons for assuming that both are related to each other." On the other hand Vedder and Chamberlain<sup>17</sup> said that both are essentially identical.

Besides the argument of identity of these two diseases, the metabolism of the human body is different from that of birds. In birds the urea is replaced by uric acid. One may say that without similar experiments or observations on man the results obtained with fowls would not be applicable to man.

Let us consider the observations upon the production of beriberi in the human body. Fletcher<sup>18</sup> states: At the commencement of the experiment all patients showing symptoms of beriberi were removed to the district hospital. The result was that 34 out of 120 persons fed on uncured (Siamese) rice suffered from beriberi and 18 died, while among 123 patients dieted on cured rice there were no deaths from beriberi. Kiewiet de Jouge<sup>11</sup> made

experiments on 384 patients. Katjang idjo was given to 182 of them, but not to the remaining 202. The result was as follows:

	With katj. idjo (per cent)	Without katj. idjo (per cent)
Suffered from beriberi and remained unchanged	15.0	23.4
Improved	75.0	13.3
Became worse	10.0	63.3
Died	2.5	30.0

Takaki succeeded in eradicating beriberi from Japanese Navy by allowing a large portion of nitrogenous food; he believes that beriberi is due to nitrogen starvation, that is, the disproportion of nitrogenous and nonnitrogenous elements. As to the nitrogen equilibrium many experiments were made in different laboratories. The average of 109 experiments belonging to twenty-five investigations<sup>19</sup> showed an indicated requirement of 0.635 gm. of protein per kilo of body weight. It was estimated that the diet given to the people in Bilibid prison consisted of: 97.17 gm. of proteins, 17.24 gm. of fats, 491.04 gm. of carbohydrates and 26.52 gm. of salts. Yet the outbreak<sup>9</sup> of beriberi in that prison was not prevented. On the other hand, there were no deaths from beriberi in Culion, where the deaths from beriberi in previous years were 309, during the interval following the use of unpolished rice.<sup>20</sup>

Not only the theory of nitrogen starvation may be disproved by these observations, but also the deficiency in some particular,<sup>21</sup> growth-promoting amino-acids may be excluded. The chief protein of rice is oryzenin,<sup>22</sup> a glutelin. Fuji and others<sup>23</sup> have determined some of the amino acid cleavage products of this protein. The analyses indicate that the protein is regarded as "complete." Moreover human beriberi may also be caused by diets which do not include polished rice, as shown from the observation of Axel Holst,<sup>24</sup> of Little,<sup>25</sup> and of Lavolaee.<sup>26</sup>

With reference to the nature of the influence of rice causing beriberi some theories other than vitamin deficiency may be mentioned. Braddon<sup>29</sup> concluded: That beriberi was not an infectious disease but a form of food poisoning. He suggested that the cause was not indeed rice, but diseased rice, rice with a poison derived from decay due to some fungus, or mould, or spore. Considering Braddon's idea Fraser and Stanton carried out experiments in an isolated district. The blood and urine of patients suffering from beriberi were examined, but in no instances were special organisms found. The results lend support to the view that beriberi has at least a relationship with the consumption of a certain particular sort of rice. Eykman concluded<sup>13</sup> that a poison existed in rice and for this poison something in the pericarp was an antidote. After arriving in Holland he made some more experiments and again concluded<sup>27</sup> that the poison was present in starch or developed from it. Eykman's poison theory was disproved by Grijns, who succeeded in causing polyneuritis in fowls by feeding them on food without starch, and concluded that the nerve system requires unknown constituents of food. He injected into the peritoneal cavity of fowls the blood of diseased



birds (a maximum of 220 cubic centimeters in 23 injections), but with negative results.

"Phosphorus starvation" is another theory in regard to the etiology of beriberi. Aron<sup>28</sup> concludes that foodstuffs which are poor in phosphorus (phytin) have been shown to cause beriberi. With reference to phosphorus requirement of man, the survey made by Sherman<sup>30</sup> deserves notice. The data from ninety-five experiments range from a minimum of 0.52 gm. to a maximum of 1.20 gm. with an average of 0.88 gm. phosphorus per 70 kg. daily. The minimum requirement of phosphorus is rarely approached. Moreover Simpson and Edie<sup>31</sup> found that the disease was neither cured nor prevented by the organic phosphorus compounds. The relation may be such, that rice with a phosphorus pentoxide ( $P_2O_5$ ) content below 0.4 per cent is deficient in vitamins too, as concluded by Stanton and Fraser.<sup>32</sup>

It is not merely a theory but a fact that deficiency in certain substance, "vitamin" (or vitamine) is, if not the only one, at least one factor in causing beriberi. For there are so many feeding experiments, clinical observations, and chemical investigations which lend the same support to the vitamine causation, that it seems to be unnecessary to quote them in detail. Funk<sup>33</sup> has discovered a method to isolate the alcohol-soluble protective substance from yeast, rice-polishings and other stuffs. He considered this substance a pyrimidine derivative. In addition to Funk's base Vedder<sup>34</sup> has reported the isolation of two groups of substances (purine bases, choline-like bases) from rice-polishing, which are capable of partly protecting fowls. Both Funk's base and Vedder's bases are found in undermilled rice, but the starchy part of cereal food is poor in the "antineuritic vitamine." This explains the clinical cases as well as the feeding experiments. In Fraser and Stanton's study, beriberi appeared among the members of the party receiving polished rice but not among the party eating undermilled rice; after reversing the food of both parties the conditions were reversed.

Clinical cases give us no less convincing explanation than the laboratory experiments. Heiser<sup>35</sup> reports 50 cases of beriberi cured (except two very advanced cases) by giving daily 15 grams of rice polishings. Danier<sup>36</sup> at Sargon reports the efficiency of the preventive use of rice-bran. William and Saleeby<sup>37</sup> state that in 5 of 6 cases in which vitamine was prescribed, improvement was very radical. These are in general accord with the results on Chinese soldiers, as I have noted in the Chekiang Army Hospital.

As to the treatment and prevention of beriberi, there are nearly as many theories as concerning its etiology. Our very ancient people, believing the theory of "Chee-Nih" (improper antagonism of gas in circulation), developed a custom as a means of prophylaxis. They thought that "nien-wei" or "nooh" (a kind of fancy dressing parade), arousing, stimulating and amusing the mass of people, might relieve and neutralize the ill effect of inactive and depressive life, which was supposed to cause "Chee-Nih." However, some of them knew that "nooh" would be carried out more efficiently if the common people believed it to be God's will. That is why "nooh" was considered religiously and its purpose of preventing beriberi and other diseases,

as proposed by a few of the ancient initiators and described in an old book, "Chu-Ree," was not understood.

Since various methods to extract vitamins from rice-polishings and other stuffs have been developed, the etiology, the treatment and the prevention have gained a further step. The administration of Funk's base (Funk's vitamin) is followed by prompt improvement,<sup>34</sup> which was not known before Funk's work. The administration of unhydrolized extracts obtained from 1 kilogram of rice-polishings results in the immediate dissipation of edema. The case of infantile beriberi (of the "wet" type) has been cured<sup>34</sup> by giving that portion of extract which does not contain Funk's base but contains bases of other kinds. If the autolyzed yeast extract is used, adults are given from 15 to 40 c.c. three times a day; children may be given from 2 to 4 c.c. every three hours.<sup>38</sup>

#### DISCUSSION

Comparing what has been done by others and what I have seen in Chekiang the following hypothetic possibilities may be considered. The minimum vitamin, i.e., such amount of vitamin that is necessary to maintain normal metabolism without showing beriberi symptoms, probably differs in individuals. In the same individual it may vary in different tissues, and may be different from time to time when condition and metabolism are changed. The beriberi symptoms would appear when one or several of the following conditions should occur:

- Increase in the elimination or destruction of the specific vitamin (or vitamins).

- Decrease in the intake of vitamins.

- Decrease in the intake of vitamin-building materials (if the body cell can manufacture vitamins).

- Attachment of vitamin to certain tissue and thus retained, or rendered inactive by combining with other substances.

- Decrease in the capability of body cells to produce vitamins.

- Decrease in the capability of body cells to utilize vitamins.

- Increase in the demand of vitamin so that the normal minimum amount is not sufficient to meet the need.

Under certain conditions the beriberi symptom does not appear even if the vitamin content in the body is below the normal threshold. For instance, the administration<sup>39</sup> of calcium lactate prolongs the period required for the development of neuritis. This is probably due to the fact that in the presence of calcium lactate the vitamin may be used up to a degree below the normal minimum. Clark believes that this has permitted degeneration to occur in certain fibers of the sciatic nerve to a degree much more intense than has been observed for fowls fed on rice alone.

We do not know the nature of vitamin action in normal metabolism or in beriberi. Neither do we know to what extent vitamins are catabolized and eliminated daily. Some believe that they play a rôle which might be likened to that of the hormones in the cellular activity or the opsonins and other antibodies in the infection. Veogthin and Myers<sup>40</sup> say that extracts

obtained from brewers' yeast cause an increase in pancreatic secretion when injected intravenously. Secretin solutions obtained from the duodenum of the hogs contain a considerable amount of antineuritis vitamine, as shown by their power in relieving the paralytic symptom of polyneuritic pigeons. Starling points out that hormones should either be easily destroyed or readily excreted. Their destruction is influenced by different conditions. Although the identity of the water-soluble vitamine B and secretin has been denied,<sup>50</sup> it is not impossible that the destruction of vitamine also is influenced by some factors.

Either decrease in the vitamine-building material or in the capability of body cells affects the manufacturing of vitamins in the body. It is not known whether the human body-cell can synthesize Funk's base (vitamine) from nonvitamine materials. It is known that secretin and other hormones are formed in the body.<sup>41</sup> Some claim that<sup>40</sup> the chemical and physical properties of secretin and vitamine are similar. They might be derived from histidin by splitting off carbon dioxide, or the one might be derived from the other by rearranging the atoms in the molecule. Funk,<sup>33</sup> Williams and Saleeby<sup>42</sup> report that allantoin (0.1-0.4 gm. three times daily) has a beneficial effect on certain cases of beriberi. The latter suggests that allantoin resembles Funk's vitamine in its chemical constitution. From these statements it appears to be that either the body cells manufacture vitamins as they do in the cases of secretin and allantoin, or they use them as raw materials. It also appears to be not improbable that normally beriberi does not occur, because the vitamine equilibrium is maintained by two sources: exogenous and endogenous. If one of the two, under certain conditions, is diminished and the other is not sufficient to compensate it, then the vitamine in the body is below its normal minimum (safe minimum) and beriberi occurs.

In the fall of 1916, there were about two hundred beriberi patients in the army hospital, when no beriberi cases appeared in the school hospital in the same city. The sanitary condition of soldiers was better than that of the civil laborers. Soldiers did feed on undermilled rice, vegetables and meat. Yet the outbreak was not prevented. As far as food was concerned, the vitamine content would have sufficed them if they had been in civilian life. As they had to take drill, their metabolic demands might be changed. Thus, standing in a rigid military position is said to increase creatinine excretion.<sup>43</sup> Vedder and Clark<sup>44</sup> state that chromatolysis and changes in the tigroid substance of the cells of the cord, similar to that of the pigeons which have been exhausted by long flights, may also be demonstrated in birds which have developed polyneuritis. This shows that muscular work affects the nerve system somewhat as the beriberi does, probably by exhausting vitamins. Soldiers, doing muscular work, possibly demand more vitamins; consequently the normal vitamine content of civilian life is not sufficient to meet the increased need of the soldier and thus to prevent beriberi. From the experience of the outbreak of beriberi in Kut, Hehir<sup>45</sup> concludes that the onset of beriberi was precipitated or accentuated by marching or any kind of severe continuous exercise. This may also be proved by the facts in Chekiang First



Division. A number of cases were checked either by allowing a rest before the soldier became exhausted or by giving him more vitamins than necessary for civilian life. We found that, although we did use undermilled rice, the vitamin content was insufficient to meet the increased demands. After giving a certain amount of unpolished rice and wheat and rice-bran, good results were shown.

It seems probably that the mental factor might play a no less important rôle in vitamin variation than the drill. The psychologic excitement might influence the vitamin metabolism by modifying the ability of body cells. By increasing a certain kind of internal secretion or by other means vitamin in the body might be destroyed more rapidly and abundantly; or, through a molecular rearrangement or a firmer union, its action might be changed and would not be utilized as usual. Or during mental excitement the nervous system or the body cells as a whole, being affected by internal secretions or other means, might lose the power of forming and utilizing the vitamins economically. In the case of emotional diabetes, for instance, mental excitement brings about an abundant output of adrenaline and an increased destruction of glycogen. McCarrison<sup>49</sup> has found that the suprarenals of beriberi patients undergo a marked hypertrophy. Sometimes the body cell may lose the ability of utilizing certain substances: in the absence of pancreatic internal secretion the body cells lose their capability of utilizing carbohydrates. Furthermore, it may also be shown by the following fact. Hight<sup>46</sup> reported that a Siamese police sergeant, who, having had a dispute with one of his subordinates, was put into a police cell to await trial. In thirty-six hours he became so ill that he was transferred to a hospital where he died in a few hours, with all the symptoms of acute, wet beriberi. In the winter of 1916 the term "char-chee" beriberi) was so familiar to the soldiers of the First Division that nearly a "beriberi-phobia" was in existence. There were three men who, doing some writing work, did not have drill, suffered from beriberi. As they were in a condition of fearing beriberi infection, their vitamin metabolism might be changed owing to the mental uneasiness.

Steenbock and Bontwell<sup>47</sup> have obtained evidence that under certain conditions the vitamin is very labile and under other conditions it may be stable. Williams<sup>48</sup> succeeded in preparing, synthetically, substances that would cure polyneuritis. These substances display a dynamic isomerism which has a profound influence on their antineuritic properties. The curative force of each compound readily undergoes at ordinary temperature a rearrangement of its atoms resulting in the formation of physiologically inert substance. By application of dry heat the noncurative form can be reconverted into a curative one. Although the synthetic substance in which this physiologically important isomerism was first discovered was hydroxypyridine, it now seems probable the antineuritic properties are not peculiar to them but are inherent in the type of isomerism of which they are capable. Such laboratory work has shown the probability that the utilization and activity of vitamin in the body might be diminished under certain conditions.

Deficiency in vitamin metabolism is not always accompanying the defi-



ciency in general nutrition. The body weight of our beriberi soldiers, at the beginning of the disease, was not less than when they enlisted. Their vital resistance was not always lowered, at least not at the beginning. This was shown by the fact that the majority of our beriberi patients did not have erosion about the corners of the mouth. While in the school hospital (where clinical training is given to medical students) our civilian patients, whose general nutrition condition was poor, as manifested by the sore mouth and tongue, and conjunctivitis, did not show beriberic symptoms.

## SUMMARY

Disturbance of vitamine metabolism is one, if not the only one, factor causing beriberi.

The disease can be produced in such a way that the infectious theory of its origin can be regarded as no longer tenable.

The theory of nitrogen-starvation or of phosphorus-starvation may be discredited by comparing with the actual requirement.

The source of vitamine in the body might be endogenous as well as exogenous.

Muscular or mental strain might increase the vitamine requirement and assists to develop beriberi.

Under different conditions vitamine may be labile or stable, active or inactive, so that the same amount of vitamine in the body may not have the same antiberiberic function.

Under different conditions the capability of cells and the utilization of vitamine in the body might be changed, which would have influence on causing beriberi.

Many other theories of beriberi etiology, besides vitamine deficiency, can be disproved by experiments.

Deficiency in vitamine and deficiency in general nutrition may not always go side by side: beriberi may appear before the general condition breaks down and may not appear even after the disturbance of general nutrition.

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# THE EFFECTS OF THYROID, THYROXIN AND OTHER IODINE COMPOUNDS UPON THE ACETONITRILE TESTS\*

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THE controversy over the question whether the thyroid exerts its influence in the animal body by secreting a substance which acts positively in certain processes of metabolism or whether its function is to neutralize poisons, led Hunt<sup>1</sup> (1905) to study the value of the thyroid in increasing the resistance of mice to poisoning with acetonitrile. He found that in mice which had received desiccated thyroid with the diet, the resistance of this poison was remarkably increased. The effect of the thyroid in some of his experiments was proportional to its iodine content, Hunt and Seidell<sup>2</sup> (1910). As a result of this work, however, Hunt went so far as to say that this protective action of the thyroid is a specific physiologic test for this tissue, more delicate than any known chemical test. The results were confirmed by Trendelenberg<sup>3</sup> (1910). On the other hand, the work of Carlson and Woelfel<sup>4</sup> (1910), Olds<sup>5</sup> (1910) and Lussky<sup>6</sup> (1912) seems to refute the claims of Hunt.

Although the active thyroids afforded protection against the poison in proportion to the amount of iodine contained, experiments of Hunt and Seidell<sup>2</sup> (1910) with a number of iodine compounds indicated that the protective action was not a function of the iodine content alone. Out of a large number of experiments, however, Hunt fed thyroid in equi-iodine dosage in only one case.

It is possible that the good results with the thyroid are the result of the amino acids of the thyroid protein supplementing the rather inadequate proteins of the wheat flour of which the mouse food of earlier workers was largely composed. That lack of vitamin or salts is not a likely factor in the susceptibility of the mice to the poison has been shown by us.

It seemed of interest, therefore, to study anew the activity of iodine in different samples of thyroid and in the other iodine compounds, including Kendall's thyroxin, in connection with the acetonitrile test. In view of the fact that on the Ehrlich cake diet, Hunt's mice lost weight, it seemed of importance to use complete diets of known nutritional value in order to obviate the possibility of a supplementing action of the thyroid proteins.

## EXPERIMENTAL PART

The following food was given in all of the experiments:—

Casein 31, brewery yeast 2, salt mixture† 5, starch 40, and butter fat 22 per cent.

That casein is an adequate protein has been shown by Osborne and Men-

\*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn. Received for publication, July 5, 1921.

†Osborne and Mendel: Jour. Biol. Chem., 1918, xxxiv, 131. The lactose and potassium iodide were omitted.

del for rats and by us for mice. On the above food all of the mice gained in weight in most of the experiments. In Table I are the results of the comparison of potassium iodide and a sample of thyroid. It will be seen that when the total amount of iodine taken as thyroid is as small as 0.0014 mg., the resistance to the poisonous action of acetonitrile is increased. With twice the dose of thyroid the resistance is doubled. When an equal amount of iodine is taken as potassium iodide, however, no increased resistance whatever is observed nor does twice or three times that amount of potassium iodide have any effect.

TABLE I

	THYROID DIET A	THYROID DIET B	I-FREE DIET	IODINE DIET A	IODINE DIET B	IODINE DIET C
Per cent iodine in substance	0.11	0.11				
Iodine in 100 gm. food (mg.)	0.055	0.110	0	0.055	0.110	0.264
Ave. daily food intake of each mouse (gm.)	2.6	2.2	2.8	2.6	2.9	2.1
Ave. daily thyroid intake by each mouse (mg.)	1.3	2.2				
Ave. daily iodine intake by each mouse (mg.)	0.0014	0.0024		0.0014	0.0032	0.0055
Number of mice	8	8	5	8	8	8
Duration of feeding (days)	9	9	9	9	9	9
Ave. change in body weight (gm.)	14.3-17.5	14.2-16.2	17.5-19.0	15.4-17.9	15.0-17.7	16.5-19.8
Minimal lethal dose acetonitrile (mg.)	1.92	3.30	0.51	0.45	0.50	0.70

In order to study the activity of the iodine in various samples of thyroid from various species of animals and to compare it with the iodine in other compounds, the following materials were used:

	Per cent Iodine
(1) Thyroid, normal, hog, summer	0.43
(2) Thyroid, normal, sheep, winter	0.06
(3) Thyroid, normal, beef, winter	0.08
(4) Thyroid, enlarged, beef, fetal (6-9 mo.)	0.06
(5) Thyroid, normal, beef, fetal (6-9 mo.)	0.28
(6) Thyroid	0.11
(7) Di-iodotyrosine	58.66
(8) Thyroxin	65.1

Thyroids (1) to (5) were obtained through the courtesy of Dr. Fenger, Armour Laboratories, and the thyroxin was kindly furnished by Dr. Kendall of the Mayo Clinic.

These materials were dried to constant weight in vacuo at room temperature in order to avoid any possible change in the physiological activity due to heat. They were then carefully triturated and incorporated in the standard diet. The di-iodotyrosine and thyroxin were mixed with starch in 0.4 per cent mixture and then used in the diet.

With di-iodotyrosine Strouse and Voegtlin<sup>7</sup> (1909) observed no thyroid-like effect on the nitrogen metabolism or on the blood pressure of normal dogs nor any favorable effect on the condition of myxedematous and cretinous animals. Morse<sup>8</sup> (1914), however, reported a positive Gudernatch test on tad-



TABLE II

	CONTROL	THYROID (1)	THYROID (2)	THYROID (3)	THYROID (4)	THYROID (5)	THYROID (6)	DI-iodotyrosine	THYROXIN
Per cent iodine in substance									
Iodine in 100 gm. food (mg.)	0	0.43	0.06	0.08	0.06	0.28	0.11	58.7	65.1
Ave. daily food intake by each mouse (gm.)	2.5	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Ave. daily intake of substance by each mouse (mg.)		2.2	2.2	2.3	1.9	2.0	2.1	1.7	2.0
Ave. daily intake iodine by each mouse (mg.)		0.85	6.05	4.75	5.23	1.18	3.15	0.003	0.005
Ave. daily intake iodine by each mouse (mg.)		0.0036	0.0036	0.0038	0.0031	0.0033	0.0035	0.0028	0.0033
Number of mice	8	8	8	8	8	8	8	8	8
Duration of feeding (days)	11	11	11	11	11	11	11	11	11
Ave. change in body weight (gm.)	15.7-18.2	14.5-14.9	14.7-15.5	14.9-14.7	15.5-17.0	15.3-16.9	16.0-16.4	15.7-17.8	15.6-16.9
Minimal lethal dose acetonitrile (mg.)	0.40	2.15	1.10	1.70	0.40	0.32	1.35	0.15	0.53

poles with this material. Thyroxin, the crystalline material isolated from the thyroid by Kendall<sup>9</sup> (1919), seems to embody all of the distinctive physiologic properties of the thyroid. He has shown that thyroxin cures myxedema and cretinism.

TABLE III

		CONTROL	DI-IODOTYROSINE	THYROXIN
Per cent iodine in substance			58.7	65.1
Iodine in 100 gm. food (mg.)			0.17	0.17
Ave. daily intake food by each mouse (gm.)	1.6		1.5	1.9
Ave. daily intake of substance by each mouse (mg.)			0.0042	0.0048
Ave. daily intake iodine by each mouse (mg.)			0.0025	0.0031
Number of mice	7		7	7
Duration of feeding (days)	11		11	11
Ave. change in body weight (gm.)	11.8-13.2		11.6-13.2	11.8-11.9
Minimal lethal dose acetonitrile (mg.)	0.23		0.25	0.37

In Tables II and III are given the results of the experiments on the protective action of these various substances. All were fed in such doses of the material that each mouse received an equal amount of iodine. From the table it is seen that all of the thyroids, with the exception of the fetal thyroids, gave marked protection against poisoning with acetonitrile. Thyroxin gave slight protection but di-iodotyrosine gave none. In the light of later experiments, the behavior of thyroxin in this experiment seems paradoxical. When the fetal thyroids were increased to ten times the amount the protection was increased but not to the extent afforded by the mature thyroids (Tables IV and V). Increasing the amount of di-iodotyrosine, however, had no effect, the mice receiving the increased dose having no more resistance than the controls. In this series of experiments (Table IV) it is noted that increased thyroxin failed to increase the resistance to acetonitrile, which seemed to be the case with the most active thyroid when they were both compared in experiments reported in Table VI. The ease of thyroxin calls for special attention.

In Table IV are given the results showing the effect of feeding three different doses of thyroxin. Here we see that, with the largest dose, the protection against acetonitrile is smaller and there is a greater loss of weight. This led to a repeated comparison of the most active thyroid and thyroxin both being fed in doses of 0.003 mg. iodine per mouse and in three times that amount. In this series of experiments (Table VI) thyroxin compares favorably with the most active thyroid. When milk was added to the diet to prevent the loss in weight, if possible, the animals fed thyroxin not only stopped losing weight but gained more than the thyroid-fed animals. These experiments (Tables IV and VI) also show what a remarkable effect thyroxin has on the metabolism in the organism, for when thyroxin was fed in quantity approaching that of the active thyroid, the metabolism of the animal was speeded up to the point where it lost weight and this loss of weight was roughly proportional to the amount of thyroxin fed. The failure of thyroxin to protect the animals in earlier experiments (Tables II and III) may be due to the low resistance of this lot of animals to the relatively large dose of thyroxin, they being fed sixty times more than the therapeutic dose for man.

TABLE IV

	CONTROL	THYROID (4)	THYROID (5)	DI-iodoty- rosine	DI-iodoty- rosine	THYROXIN	THYROXIN	THYROXIN	IODINE FOOD (KI)	UNDER- FEEDING
Per cent iodine in substance Iodine in 100 gm. food (mg.)		0.06	0.28	58.7	58.7	65.1	65.1	65.1	76.4	
Ave. daily food intake of each mouse (gm.)	1.9	2.0	2.4	1.9	1.9	1.9	1.5	1.6	1.8	1.6
Ave. daily intake of substance by each mouse (mg.)		55.0	13.8	0.0053	0.026	0.0041	0.011	0.024	0.023	
Ave. daily iodine intake by each mouse (mg.)		0.033	0.039	0.0031	0.015	0.003	0.007	0.015	0.017	
Number of mice	8	8	8	8	8	8	8	8	5	
Duration of feeding (days)	11	11	11	11	11	11	11	11	11	
Ave. change in body weight (gm.)	17.9-18.7	18.3-18.8	18.7-17.3	18.5-17.9	18.7-17.7	18.8-17.5	18.4-15.4	18.4-14.8	18.2-18.3	19.9-18.7
Minimal lethal dose acetonitrile (mg.)	0.34	0.91	2.34	0.29	0.21	0.624	0.57	0.56	0.34	0.37

Before further discussion, it is important that we have a summary of the experimental facts before us. The protective action of the thyroid is not a function of the iodine content alone for potassium iodide or di-iodotyrosine when given by mouth does not protect mice from poisoning with acetonitrile. Thyroids vary greatly among themselves in their ability to give resistance to acetonitrile poisoning—the fetal glands being least valuable in this respect. Although Hunt reported that the active thyroids gave resistance to acetonitrile in proportion to their iodine content, the most active thyroid tried by us did not behave in this way. Thyroxin, in the smallest dose used, may be as potent as the most active thyroid; in increasingly large doses it not only lowers the resistance of the animal to acetonitrile but also causes loss in weight.

TABLE V

		CONTROL	DI-IODOTYROSINE	THYROID(5)	THYROXIN
Per cent iodine in substance			58.7	0.28	65.1
Iodine in 100 gm. food (mg.)			0.825	1.7	0.17
Ave. daily food intake by each mouse (gm.)	2.4		2.3	2.6	2.6
Ave. daily intake of substance by each mouse (mg.)			0.032	14.99	0.0065
Ave. daily intake iodine by each mouse (mg.)			0.019	0.04	0.004
Number of mice	5		4	8	6
Duration of feeding (days)	7		7	7	7
Ave. change in body weight (gm.)	20.2-20.5		18.6-18.7	17.2-16.7	19.3-18.4
Minimal lethal dose acetonitrile (mg.)	0.60		0.24	4.8	4.0

TABLE VI

	CONTROL	THYROID (1)	THYROID (1)	THROXIN	THROXIN	THROXIN + MILK
Per cent iodine in substance		0.43	0.43	65.1	65.1	65.1
Iodine in 100 gm. food (mg.)		0.17	0.495	0.17	0.495	0.17
Ave. daily food intake per mouse (gm.)	1.81	2.09	2.06	2.08	1.79	1.7
Ave. daily intake of substance by each mouse (mg.)		0.80	2.37	0.005	0.013	0.004
Ave. daily intake of iodine by each mouse (mg.)		0.003	0.01	0.003	0.009	0.003
Number of mice	7	8	8	8	8	6
Duration of feeding (days)	10	10	10	10	10	10
Ave. change of body weight (gm.)	16.5-18.9	16.3-17.1	16.4-17.4	16.5-17.6	16.1-14.7	15.1-16.6
Minimal lethal dose acetonitrile (mg.)	0.45	6.4	6.0	5.6	5.2	6.0

Why do the various thyroids differ in their ability to protect mice against acetonitrile? It is not due to the iodine content for they were all fed in equi-iodine doses. It is probably not due to a species difference, for the beef thyroids differed among themselves. It may be that in the preparation the material was rendered inert,—a possibility but not a probability. Or it may be that the iodine is combined in varying ratios of active and inactive forms. In the light of Kendall's<sup>10</sup> (1915) recent work the latter hypothesis seems to be applicable. According to him all of the iodine of the thyroid can be divided by alkaline hydrolysis into two forms: the "A" form, comprising one-half of all the iodine, insoluble in acid, curing myxedema and cretinism and from



which thyroxin was isolated, and the "B" form, comprising one-half of the iodine, acid soluble but almost inert physiologically. In another place Kendall<sup>11</sup> (1917) states that the "A" form may vary from 2 to 3 per cent to 40 to 50 per cent which might help explain some of our results.

TABLE VII

	CONTROL	THYROID (1)	THYROXIN
Per cent iodine in substance	0	0.43	65.1
Iodine in 100 gm. food (mg.)		0.17	0.06
Ave. daily food intake of each mouse (gm.)	1.86	1.8	1.8
Ave. daily intake of substance by each mouse (mg.)		0.69	0.002
Ave. daily iodine intake by each mouse (mg.)		0.003	0.00099
Number of mice	6	6	6
Duration of feeding (days)	11	11	11
Ave. change of body weight (gm.)	16.3-18.0	16.1-17.3	16.1-16.8
Minimal lethal dose acetonitrile (mg.)	0.48	2.08	3.20

If the ratio of "A" iodine to "B" is 1:1 in the thyroids it may be that we have been feeding thyroxin in too strong a concentration, since it corresponds closely to the "A" form and we fed our material in equi-iodine amounts. Therefore the experiments recorded in Table VII were performed, with the result that when thyroxin was fed in one-third the amount of the most active thyroid preparation (based on the iodine content) the protection against acetonitrile was greater than that given by the thyroid. This seems to point to the possibility that it is the "A" iodine in the thyroid which is concerned in producing the resistance to acetonitrile and that thyroxin, which is obtained from this fraction, contains the protective substance in very high concentration.

Kendall<sup>12</sup> (1918) states that the thyroid, through the action of its hormone, determines how much energy a given cell can produce on stimulation from without or within. In other words, the thyroid hormone is an important link in physiologic oxidation. He also states that the imino group is the one in thyroxin which is concerned in the metabolic effects and that the iodine only serves to make the imino group more reactive. That the iodine has no effect upon metabolic rate has been shown by Kendall<sup>13</sup> (1919) in experiments in which the imino group of the thyroxin was substituted, but he was able to influence the metamorphosis of frogs with this material the same as when the imino group was unchanged. Which group in thyroxin is responsible for the protective action against acetonitrile? The fact that thyroids vary in their protective action so markedly when fed in equi-iodine amounts, the fact that iodine-free thyreoglobulin (Hunt<sup>14</sup> 1907) produces a similar effect to that of iodine-containing thyroids, the minute amount required compared to that of other iodine-containing compounds all indicate that the protective action of the thyroid depends upon the metabolic effect of its active principle rather than upon the iodine contained therein.

On the other hand, it is known that acetonitrile produces its bad effect through its oxidation to hydrocyanic acid. If, now, the beneficial effect of thyroxin is due to its tonic effect on the normal cell which ultimately means facilitated oxidation, it seems difficult to understand the mechanism whereby a

substance promoting oxidation can protect an organism from a reaction depending on oxidation for its harmfulness. Although the present investigation has not disclosed the ultimate mechanism of thyroid action, it indicates that thyroxin is indeed an active principle of the thyroid which increases the resistance of mice to acetonitrile.

#### SUMMARY

(1) Desiccated thyroid fed to mice protects them against poisoning with acetonitrile. The thyroid seems to be efficient in proportion to the amount of iodine contained.

(2) Potassium iodide and di-iodotyrosine gave no protection against acetonitrile in whatever quantity fed.

(3) Thyroxin when fed in such an amount that it furnished one-third the iodine provided by the most active thyroid fed, in some cases gave a greater degree of protection. Increasing the amount of thyroxin, decreased the protection against acetonitrile and caused loss of weight of the animals.

The author wishes to express his gratitude to Professor Lafayette B. Mendel for his helpful advice and criticism.

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# LABORATORY METHODS

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## AN ADAPTATION OF THE FOLIN AND HU BLOOD SUGAR METHOD APPLICABLE TO SMALL AMOUNTS OF BLOOD\*

### A COMPARISON OF THE BLOOD SUGAR CONTENT OF VENOUS AND CAPILLARY BLOOD

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IT is very desirable and in fact often necessary to estimate the blood sugar by some procedure requiring only the small amounts of blood that may be obtained from pricking the finger. Various micro methods of this kind have been used, the Bang,<sup>1</sup> adaptations of the picric acid method of Lewis and Benedict<sup>2</sup> and Déhu and Hartman,<sup>3</sup> and the MacLean,<sup>4</sup> being among those more commonly employed. The Bang procedure while long and most exacting, is so apt to give erroneous results that analyses must be done in triplicate. The picric acid method of Epstein<sup>5</sup> has often been found to yield results considerably higher than those obtained by other procedures and we have had similar experiences with it. Like all colorimetric methods in which the color measured is the resultant of two or more colors, in this case sodium picrate and sodium picramate, serious errors are often introduced as shown by Falk and Noyes.<sup>6</sup> The work of the Harriman Research Laboratory investigators has raised a serious objection on both theoretical and practical grounds, to the use of any picric acid reduction method, where another procedure is available. The MacLean method seems to have yielded excellent results though it is quite long and great care is said to be necessary.

When the systematic methods of blood analyses of Folin and Hu<sup>7</sup> were published, they were tried out in this laboratory, and after thorough testing, found to be very satisfactory, and adopted as our standard procedures. With a view to keeping our methods limited to trustworthy ones, and as few as possible, we endeavored to adopt the blood sugar method of Folin and Hu for an amount of blood that could be obtained by pricking the finger. This we have succeeded in doing in a very satisfactory way, using 0.4 c.c. of blood. The adaptation is comparatively simple and rapid and is free from the objections that have just been pointed out. It has been in use here and at other laboratories for the past two years. The procedure is as follows:

Ordinary one c.c. serologic pipettes, graduated to the tip, in 0.01 c.c. are cut off at about the 0.6 c.c. mark and calibrated to the 0.4 c.c. mark. The pipette is connected with a rubber tube, on the end of which a small glass mouthpiece is placed. Blood is obtained, usually from the fourth, occasionally from the middle finger of the left hand by pricking with a Hagedorn needle.

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Received for publication, July 22, 1921.

Gentle pressure is applied if necessary, not too near the wound, until a large drop of blood has collected. This is carefully drawn up the pipette, in the beginning mainly by capillarity, but after about 0.2 c.c. of blood has been obtained, very gentle suction must be applied to the glass mouthpiece. The surface of the skin is kept quite dry with sterile sponges to keep the blood in a deep round drop; if the skin is not dry the blood will spread.

After 0.4 c.c. of blood has been obtained, it is immediately discharged into a 5 c.c. tube graduated in 0.1 c.c. containing about one c.c. of distilled water. The corpuscles settle to the bottom of the tube and a clot will form unless the tube is gently rotated. Frothing should be avoided. The pipette is then quickly washed with water several times, the washings added to the tube and the total volume brought to 3.2 c.c. Four tenths of a c.c. of ten per cent sodium tungstate and an equal volume of 0.7N sulphuric acid are added successively, the tube is closed with a cork or rubber stopper, shaken vigorously and allowed to stand ten or fifteen minutes. Then centrifuge in the same tubes for ten or fifteen minutes at a high speed. Filter the supernatant liquid through a very small filter ( $4\frac{1}{4}$  cm.) into a 15 c.c. or 30 c.c. beaker.

Two c.c. of the filtrate are pipetted into a Folin sugar tube and Folin and Hu's procedure followed exactly as in the macro method. That is, 2 c.c. of standard sugar solutions, equivalent to 100 and 200 mgs.\* of glucose per 100 c.c. respectively, are pipetted with Folin-Ostwald pipettes into similar sugar tubes. To both the unknown and the standards, 2 c.c. of the faintly alkaline copper tartrate solution are added and all heated simultaneously in a boiling water-bath for exactly six minutes. The tubes are then quickly plunged into cold water and kept there for two minutes, after which two c.c. of the molybdate phosphate solution are added to each. The tubes are shaken vigorously, allowed to stand about five minutes, made up to 25 c.c. and matched. Not more than four tubes should be heated at once unless more than one person can be at hand so that the molybdate phosphate solution can be added nearly simultaneously to all the tubes. The color remains practically unchanged for more than one hour.†

A comparison of results obtained by the micro and macro methods is presented in Table I.

\*Containing 0.2 and 0.4 mg. of glucose respectively.

† For convenience, the preparation of reagents as described by Folin and Hu is given:

*Ten per cent Sodium Tungstate.*—Purchased from the Vanadium Corporation of America, Primos, Delaware Co., Pa. Labelled Primos Brand and C. P.

*Alkaline Copper Tartrate Solution.*—Dissolve 40 gms. of pure anhydrous sodium carbonate in about 400 c.c. of water and transfer to a liter flask. Add 7.5 gms. of tartaric acid and when the latter has dissolved add 45 gms. of crystallized copper sulphate. Mix and dilute to one liter. If the reagents used are impure a sediment of cuprous oxide may separate in the course of one or two weeks. Should this occur, remove the clear supernatant fluid with a siphon or filter through a good quality filter paper. To test for the absence of cuprous copper, in the solution, transfer 2 c.c. to a test tube and add 2 c.c. of the molybdate phosphate solution; the deep blue color of the copper solution should almost completely vanish.

*Standard Sugar Solutions.*—Three standard sugar solutions should be on hand: (1) a one per cent pure glucose solution, which is kept as a stock solution, preserved with a thin layer of toluene or xylene; (2) a solution containing 1mg. of glucose per 10 c.c. (5 c.c. of stock solution diluted to 500 c.c.); (3) a solution containing 2 mg. of sugar per 10 c.c. (5 c.c. of stock solution diluted to 250 c.c.). The diluted solutions are also preserved with a little toluene or xylene. The diluted solutions will not keep for much more than a month in warm weather, but the stock solution should keep indefinitely.

*Molybdate Phosphate Solution.*—Transfer to a liter beaker 35 gms. of molybdic acid and 5 gms. of sodium tungstate. Add 200 c.c. of 10 per cent sodium hydroxide and 200 c.c. of water. Boil vigorously for 20 or 40 minutes so as to remove nearly the whole of the ammonia present in the molybdic acid. Cool, dilute to about 350 c.c. and add 125 c.c. of concentrated (85 per cent) phosphoric acid. Dilute to 500 c.c.



TABLE I

	COMPARISON OF MACRO AND MICRO METHODS		
	MACRO	MICRO	REMARKS
Mrs. P.	174	174	Diabetes
A. B.	105	105	
A. I.	150	155	After lunch
Mrs. H.	320	323	Diabetic coma
Dog 1	100	96	
Dog 2	132	130	During absorption
Dog 2	241	239	1 1/2 hrs. after glucose administration
Sheep 1	75	77	

In Table II the results of analyses of blood, drawn successively from the median cephalic vein and finger tip are given. After the blood was drawn from the arm, it was immediately taken to the laboratory and precipitated according to the method of Folin and Hu. As soon as blood had been drawn from the vein, specimens were obtained from the finger tip and analyzed by the method just described.

TABLE II

	COMPARISON OF BLOOD SUGAR CONTENT OF VENOUS AND CAPILLARY BLOOD	
	VENOUS BLOOD	CAPILLARY BLOOD
	MG. PER 100 C.C.	MG. PER 100 C.C.
X. B.	100	107
Mrs. P.	129	139
M.	98	108
H. S.	104	101
O. L.	92	96
? W.	120	123
? K.	101	103
B. J.	246	247
E. J.	386	390
? R.	94	99
D. C.	135	142
D. C.	117	121
L. R.	111	106
? R.	119	110
L.	92	96
Dr. A.	100	103
W.	128	128
W. D.	103	102
M. S.	106	102
S. S.	94	95
D. C.	114	110
	105	95
S. W.	90	95
H. P.	139	136
M. G.	132	134
F. I.	106	107

As might be expected on theoretical grounds, the capillary blood usually contained very slightly more reducing substances than venous blood. However, these variations may have been due to a little glycolysis of the venous blood. Occasionally on account of the distance from the wards as much as ten or fifteen minutes elapsed between the time the blood was drawn and the proteins precipitated, while in the case of the micro determinations, the precipitations were made at once.

Glucose solutions added to blood were in every instance recovered quantitatively by the above described micro method.

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## A NOTE ON THE STERILIZATION OF SURGICAL INSTRUMENTS\*

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IN a brief article written by Dr. Hibbert W. Hill, *Journal of Applied Microscopy*, Vol. iii, No. 8, the author describes the following method for sterilizing instruments during Bacteriological Autopsy work: The blades, knives, scissors, forceps, etc., are dipped into methyl alcohol and then quickly passed close enough to a bunsen flame to ignite the alcohol. The alcohol burns off instantly, leaving a dry, sterile surface free from any disinfectant.

The advantage over direct flaming lies in the tendency of the latter process to destroy the temper of the instrument. The advantages over the use of disinfecting solutions, as carbolic acid, etc., are: (1) No disinfectant is introduced into the incised tissue by the knife; (2) Sterilization is instantaneous and complete, whereas, disinfectant solutions require some appreciable time to kill the common pathogenic forms, and a considerable time to kill spores; (3) If the knife has already been used and sterilization is required, the adherent blood or tissue is readily cleared and rendered sterile, while a disinfectant solution requires time for penetration. Experimentally, also, it has been found that anthrax spores smeared upon a knife blade failed to develop when transferred to media after the blade had been flamed once in alcohol, as described above.

It appeared to the writer that the "time" in which it took to burn off alcohol† from a smooth surface was really insufficient to completely sterilize it, especially if organisms were present in the free spore stage. Hence, it seemed desirable to further investigate this method of sterilization, and if it

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Received for publication, July 5, 1921.

†Ordinary 95 per cent alcohol.

proved inefficient to determine whether or not any other inflammable fluid will accomplish the sought-for end. With these two aims in view, numerous experiments were carried on, the results of which constitute the basis of this article.

It seemed logical to begin with organisms of the nonspore-forming type, and consequently, the following representatives were chosen from the stock cultures of this laboratory: *Bact. coli*, *Ps. pyocyanea*, *Staph. aureus*, *Staph. albus*, *Strep. pyogenes*, and *Proteus vulgaris*.

For lack of sufficient sealpels to do this work on a large scale, glass rods, as advocated by Dr. Hill for disinfection experiments, were also employed. Yet, insofar as the present results indicate, there was no difference whether a glass or metal surface was employed.

Sterile glass rods were dipped into saline emulsions of the above-mentioned organisms and then placed each into a sterile test tube and allowed to dry. This accomplished, the rods were dipped, one by one, into methyl alcohol and passed close enough to a flame to ignite them. Each in turn was then rubbed over the surface of an agar slant, withdrawn, and quickly planted into a tube of plain bouillon. In all cases, rods containing the emulsion, but unburned, were run as controls. Agar and broth tubes were incubated at 37° C. and results noted at the end of 24 hours.

With these nonspore-bearing organisms no growth was ever obtained, either in broth or on agar, with rods which had been burned off, even after several days of reincubation. Controls always yielded positive growth.

Convinced that this method of sterilization, at least when employed on clean surfaces, is effective for nonspore-forming organisms, attention was next directed to members of the spore-bearing group. The following representatives were used from among the laboratory stock cultures: *B. cereus*, *B. anthracis*, *B. tetani*, *B. botulinus*, *B. sporogenes*, *Reading Bacillus*. Since this group comprises both aerobes and anaerobes, slight variations in the procedure were necessary. With *B. cereus* and *B. anthracis*, emulsions were made in sterile saline solution from old agar cultures which upon microscopic examination indicated a preponderance of free spores. As before, sterile glass rods were dipped into the emulsions and allowed to dry in sterile test tubes. The rods in turn were immersed in methyl alcohol, ignited, drawn over agar slanted in tubes, planted finally into plain broth, and incubated at 37° C. After 24 hours abundant growth never failed in the numerous times that these experiments were repeated. All controls gave positive growth also.

As for the anaerobes, old cultures growing in the egg-meat medium employed by Rettger, were used, the presence of spores having first been confirmed by a microscopical examination of each culture. Sterile glass rods were dipped into these cultures and then replaced within sterile test tubes and time given to allow the greasy material to firmly adhere to the rods. This accomplished, each in turn was immersed in methyl alcohol, ignited, planted into a tube of egg-meat and incubated at 34° C. In no instance did characteristic, abundant growth fail to take place either in tubes from rods which had been burned off, or in the controls which had not been so treated.

From the foregoing results, it seems logical to conclude that the short interval of time consumed in the burning off of a glass rod with methyl alcohol is insufficient to sterilize the surface when spores of the organisms here employed are present.

Having found that methyl alcohol in itself was inadequate, the question arose as to whether there was any liquid, which, when applied to a surface and ignited, would completely sterilize it, and, in case of an instrument, leave a clean and uninjured surface. To determine this, mixtures of different fluids with both methyl and ethyl alcohol were tried. They were as follows:

- |  |        |
|--|--------|
| 1. Methyl alcohol plus formalin          | (3:1). |
| 2. Methyl alcohol plus kerosene          | (3:1). |
| 3. Methyl alcohol plus benzol            | (3:1). |
| 4. Ethyl alcohol plus formalin           | (3:1). |
| 5. Ethyl alcohol plus 5 per cent phenol. |        |

As in previous experiments, the infected rods or scalpels were dipped into these solutions, ignited and planted into media; but with the exception of the mixtures Nos. 1 and 4, the results proved to be no better than those obtained when alcohol alone was used. Furthermore, in the case of kerosene and benzol, the burnt surface was always coated with a carbon deposit. With the alcohol formalin mixtures, no growths were obtained, and so the methyl alcohol formalin solution was selected to be employed in further investigations. Using the spores of *B. anthracis* and *B. cereus*, some thirty-five tests were made, and from these growth developed in none but two (two colonies on an agar slant in one instance, and four in the other). When spore material of the previously mentioned anaerobes was tried, growth in the egg-meat medium resulted in all instances, although as compared with the controls, it required two to three days longer before turbidity or liquifaction set in. In all probability the reason for this failure to sterilize was due to the fact that the oil or grease which is deposited upon the rods in the process of immersing them in cultures of egg-meat medium interfered with the even distribution of the alcohol formalin mixture over the surface.

It was next desired to ascertain whether or not methyl alcohol plus formalin would completely sterilize when blood is present. Hence, blood was drawn from a rabbit's ear into a sterile Petri dish and mixed with a suspension containing anthrax spores. Glass rods and scalpels were smeared with this material and set aside in sterile test tubes to dry for three hours. At the end of this period, each rod was rubbed onto an agar slant as a control, and then burned off with the alcohol formalin mixture, drawn over another agar slant and finally planted into a tube of plain broth. The scalpels were drawn over one-half the surface of a divided agar plate before burning, and over the remaining half after burning (the former being the control). All controls showed growth at the end of 24 hours, while transfers from the rods and scalpels after burning developed no growth whatever after five days' incubation. Parallel to this series, blood-treated and infected rods were burned off with methyl and ethyl alcohol, the subsequent procedure being similar to that above. In all cases, growth was present at the end of 24 hours.



Having observed that the burning off of a surface with alcohol alone had no sterilizing action when spores were present, it was naturally assumed that the flame was not hot enough to accomplish the desired result in the limited time required for the alcohol to burn. In order to obtain some idea as to the intensity of the heat produced, the bulb and lower portion of an ordinary chemical thermometer were immersed in alcohol, ignited, and the maximum rise of the mercury noted. This was repeated several times, the mercury being brought back to room temperature before the next trial was attempted. For methyl alcohol, the mercury stood on an average at 69° C.; ethyl alcohol 60° C.; methyl alcohol formalin mixture 133° C. The flame in the last-mentioned test might not have been actually any more intense than the others, but it burned for a longer period of time, which after all is a very important factor in all disinfection work. In view of the fact that some of the anthrax spores used in the above experiments were able to withstand boiling for several minutes, it does not seem possible that even a temperature reaching a maximum of 133° C. in a few seconds could in itself produce sterility. Hence, it seems logical to conclude that the results obtained with the alcohol formalin mixture were due to the chemical action of formalin in conjunction with heat.

#### SUMMARY AND CONCLUSIONS

The results set forth in this paper may be summarized as follows:

(1) No growth was obtained when either glass rods or scalpels on which nonspore-forming organisms were present were burned off with methyl alcohol.

(2) Glass rods and scalpels could not be sterilized by burning them off with methyl alcohol when spores of *B. anthracis*, *B. cereus*, *B. botulinus*, *B. tetani*, etc., were present.

(3) The burning off of glass rods and scalpels with a methyl alcohol-formalin mixture (3:1) gave excellent results except in the case of the anaerobes when grease and oil were present.

(4) When absolute sterility is desired, the burning off of a surface with an inflammable fluid cannot *always* be relied upon.

# A MICRO-MODIFICATION OF THE METHOD OF BENEDICT FOR THE QUANTITATIVE DETERMINATION OF REDUCING SUGAR IN URINE\*

BY MILLARD SMITH

OF the various methods for the quantitative determination of reducing sugar in urine, doubtless none is in more general use than that of Benedict.<sup>1</sup> This method, due to its simplicity, is unusually adapted to routine clinical work and perhaps in the hands of the technician gives results which are more accurate than would other methods which might claim on theoretical grounds the advantage of greater accuracy in the hands of the experienced chemist.

However there is coming into quite common use a modification of the Benedict method which is based upon a reasonable assumption but which in its application does not conform to the requirements therein demanded. Moreover there has apparently been no attempt to verify the correctness of the modification and unfortunately this has led to the use of a method which has an appreciable degree of error.

The argument usually given in defense of the use of the modification is that it considerably reduces the cost of the determination and at the same time greatly simplifies it. In connection with the investigation of the accuracy of the procedure the writer has therefore attempted to so modify the original method that these advantages may be retained and at the same time to avoid the error which is encountered in the present modification. It has been possible to do this satisfactorily.

The modification which is being used at present can be ascribed to no one individual for it seems to have been in use independently in several laboratories and was merely a natural deduction in the interest of economy and efficiency. It consists in the use of from one to two-fifths of the original amount of Benedict's solution specified for a determination. The amount of carbonate is correspondingly reduced but the original volume is retained by the addition of 15 to 20 c.c. of distilled water. The titration is then carried out as usual in a porcelain dish.

It might seem that such a procedure would be permissible but this slight modification leads to a variable error of from 15 to 30 per cent. The explanation for the error lies in the disturbed alkalinity of the titrating solution which is caused by the dilution of the reagent with water. Even the use of the same amount of carbonate as in the original determination does not remedy the error. The buffer action of the citrate in definite concentration is evidently necessary for the proper reaction. Even with the original method a similar degree of error is encountered in the determination of a urine of low sugar

\*From the Clinical Laboratory of Dr. Elliott P. Joslin at the New England Deaconess Hospital, Boston.

Received for publication, September 9, 1921.

content, i.e. 0.2 per cent, if care is not taken to keep the volume of the titrating solution constant. This necessitates the evaporation during the titration of the entire amount of urine added, which amounts to 25 c.c. Such a determination is time-consuming and laborious. The endpoint is very difficult and unsatisfactory to read.

Another difficulty often encountered with the modified method is a precipitation of the red oxide of copper which obscures the endpoint. This is due to the dilution of the potassium sulphocyanate and potassium ferrocyanide. These two reagents are needed in good concentration to hold the red oxide in solution. However, as pointed out by Benedict,<sup>2</sup> this could be avoided by the use of more ferrocyanide in the standard solution.

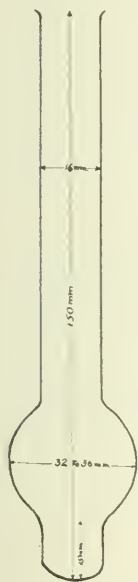


Fig. 1.

There seems to be no reason why correct results could not be obtained if the original assumption (that proportionate parts of the reagents should do as well as the full amount) was strictly adhered to. This has been done and the result has been very gratifying. The difficulty of the determination of very weak (0.2 per cent) sugar urines has largely been overcome, the endpoint is considerably sharper and the time for a determination has been reduced. This has been secured by the use of 1 c.c. of the original Benedict's quantitative solution without the addition of water but with a proportionate reduction of carbonate. The determination is carried out in a special test tube (See Fig. 1) rather than in a porcelain dish. With the use of such a small quantity of Benedict's solution evaporation would be too rapid from a dish to keep the volume during the titration constant. In the tube it is much easier to control evaporation, for with moderate boiling it is almost nil, while with vigorous boiling it is fairly rapid. The detection of the endpoint is also greatly facilitated by the use of a glass tube. The bulb is necessary to aid even boiling and to prevent too rapid evaporation and foaming.

#### REAGENTS AND APPARATUS

1. Benedict's Quantitative Sugar Reagent.
 

Copper Sulphate. 5 H <sub>2</sub> O, C.P.	18.0 gm.
Sodium Carbonate (anhydrous)	100.0
Sodium Citrate. 5½ H <sub>2</sub> O, C.P.	200.0
Potassium Sulphocyanate, C.P.	125.0
Potassium Ferrocyanide (5 per cent sol.)	5.0 c.c.

"With the aid of heat dissolve the carbonate, citrate and sulphocyanate in enough water to make about 800 c.c. of the mixture and filter if necessary. Dissolve the copper sulphate separately in about 100 c.c. of water and pour the solution slowly into the other liquid, with constant stirring. Add the ferrocyanide solution, cool and dilute exactly to 1000 c.c. Of the various constituents the copper sulphate only need be weighed with great exactness." The use of C. P. Citrate is very desirable as the U.S.P. grade gives a distinctly brownish tinge to the endpoint.

2. Sodium Carbonate (anhydrous).

3. Special test tube for titration.\* This tube is preferably but not necessarily made from Pyrex glass. An ordinary test tube of proper size and blown as in Fig. 1 will serve the purpose, but the greater mechanical strength of Pyrex glass will soon pay for the initial cost of the tubes by cutting down their breakage.

The dimensions of the tube are as indicated in the illustration. Slight variations from these dimensions are not significant but the part of the tube below the bulb should be of such size as to contain not over 2.0 c.c. or under 1.5 c.c.

4. Mohr pipette; capacity 0.4 c.c.† This pipette is to be made and graduated as follows. Distance between 0.00 c.c. and 0.4 c.c. to be between 21 and 22 cm. Tip for delivery to be about 3 cm. below lowest graduation and to be drawn to such a point that it will deliver a drop of water of not over 0.02 c.c. Upper end to be about 5 cm. above highest graduation. Pipette to be graduated every 0.02 c.c. but marked to read directly per cent sugar in urine. Markings to be every 0.02 c.c. from above downwards as follows: Per cent 100.00—10.00—5.00—3.33—2.50—2.00—1.65—1.43—1.25—1.11—1.00—.91—.83—.77—.67—.63—.59—.56—.53—.50—

5. Mohr pipette; capacity 2.00 c.c.† This pipette is to be made and graduated as follows: Distance between 0.00 c.c. and 2.00 c.c. to be between 20 and 22 cm. Tip for delivery to be about 3 cm. below lowest graduation and drawn to such a point that it will deliver a drop of water of not over 0.05 c.c. Upper end to be about 5 cm. above highest graduation. To be graduated only in per cent of sugar in urine with the graduations to come at the following points:

% graduation	c.c.
100.00	0.00
2.00	.10
1.00	.20
.70	.29
.60	.33
.50	.40
.40	.50
.30	.67
.20	1.00
.18	1.10
.17	1.20
.15	1.30
.14	1.40
.13	1.54
.12	1.70
.11	1.82
.10	2.00

6. Ostwald pipette; capacity 1.0 c.c. accurately calibrated for blow-out delivery. If desired a 1.0 c.c. Mohr pipette calibrated to deliver between two marks may be used instead of the Ostwald.

#### DETERMINATION

Accurately pipette 1.0 c.c. of Benedict's solution into the test tube (which may be held in a clamp on a ring stand) and then add 0.2 to 0.7 gm. of

\*These tubes may be purchased from the Emil Greiner Co., 55 Fulton St., New York City, for 50 cents (Pyrex) each.

†The Emil Greiner Co., will supply these pipettes for 80 cents each.



anhydrous sodium carbonate. A small, well dried pebble, or a piece of quartz, should also be added. (Benedict<sup>3</sup> suggests the use of a small piece of cotton to prevent bumping.)

Heat the mixture to boiling and add the urine from the Mohr pipette until reduction is complete as evidenced by the disappearance of the blue color. For a urine that is apt to contain 1 per cent or less sugar, the 2.0 c.c. Mohr pipette is used, and for stronger urines the 0.4 c.c. pipette. The approximate strength of the urine, with a slight amount of experience, is easily estimated from the qualitative Benedict test. It has been found that with the use of a 0.4 c.c. Mohr pipette graduated in 0.02 c.c. it is unnecessary before titration to dilute urines under 3 per cent in order to ensure sufficient accuracy. Urines over 3 per cent should be diluted to secure an accurate titration.

For *rapid* reduction of the reagent one must resort to vigorous boiling, but this may cause trouble due to too rapid evaporation and in fact it is not necessary if sufficient time is given for reduction between additions of the urine. If the solution is kept *at* the boiling point by manipulation of the flame and the urine added slowly the best results are obtained. *The tendency in the titration of sugar is to go past the endpoint. This is because the reduction does not take place as rapidly as the ordinary titrations to which one is accustomed. When nearing the endpoint the urine must be added slowly.* In urines of low sugar content the boiling should be rather vigorous at first in order to maintain a constant volume while the 1.0 to 2.0 c.c. of urine necessary to give complete reduction are being added. With a moderate amount of practice the regulation of the volume of the boiling solution becomes quite simple. A micro-Bunsen burner should be used.

#### CALCULATION

With the use of the pipettes described no calculation is necessary as the graduations read directly the per cent of sugar in the urine. However the use of these pipettes is not necessary and in their place a Folin 5.0 c.c. micro-burette for urine sugars may be used. The calculation is then:

$$\frac{0.02}{\text{c.c. urine}} = \text{per cent sugar in the urine.}$$

With urines which have been diluted the per cent obtained should of course be multiplied by the dilution.

#### DISCUSSION OF RESULTS

Table I gives a short series of determinations made by all three methods. These determinations have not been made as an attempt to indicate the greatest accuracy obtainable by either method but rather to show the accuracy to be expected when used with reasonably careful technic in the routine work of the clinical laboratory. Undoubtedly greater accuracy may be obtained by the very careful use of a Folin micro-burette and the repeating of a determination after the approximate value has been determined by a rapid preliminary titration. It will be seen in the table that the average error

TABLE I  
PER CENT REDUCING SUGAR IN URINE

URINE	BENEDICT'S METHOD	WRITER'S MODIFICATION	COMMONLY USED MODIFICATION
I	.13	.13	.10
II	.17	.17	.14
III	.36	.40	.26
IV	.37	.35	.26
V	.40	.43	.29
VI	.78	.77	.54
VII	.77	.78	
VIII	1.26	1.27	1.09
IX	1.90	2.00	1.49
X	1.98	2.00	
XI	2.69	2.50	1.90
XII	3.16	3.33	2.63
XIII	5.90	5.00	4.76
	1.53	1.47	

for the writer's modification is about plus or minus 5 per cent, the average error for the modification commonly in use being always minus about 24 per cent with variations between 15 and 30 per cent. Urine XII was titrated without diluting it and it may be seen that the titration of such a concentrated urine without dilution involves considerable error.

The titration of urines containing less than 0.17 per cent sugar is very unsatisfactory by either the original method or the writer's modification. Perhaps the most constant results (although involving a positive error of 5 to 10 per cent) is to add to the tube before boiling a good sized pinch of talcum powder. The titration is then carried out as directed and the endpoint is read while the solution is boiling. It will be white or nearly so, but when the tube stands and the talcum powder settles to the bottom it will be seen that the solution is a light dirty green. It is however better to call this the endpoint than to use no talcum powder and try to titrate until the dirty green color disappears. The writer has not found this procedure necessary for urines containing over 0.2 per cent sugar.

#### CONCLUSION

Figures are given to prove the inaccuracy of a modification of Benedict's quantitative urine sugar method now in common use.

A modification is outlined which will give correct values and at the same time greatly cut the cost and time of the determination as well as increase the ease with which the endpoint of the titration is read and the various operations are performed.

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# *The Journal of Laboratory and Clinical Medicine*

VOL. VII.

MARCH, 1922

No.

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Washington, D. C.

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## EDITORIALS

### *The Chemical Control of the Respiratory Center*

THE widespread muscular cooperation necessary for the proper performance of the acts of inspiration and expiration depends on the presence, somewhere in the brain, of a center dominating the activities of the lower spinal neurones that innervate the respiratory muscles. In order that this chief center may bring about increase or decrease in respiration according to the needs of the body, it must, as it were, be kept informed of the respiratory requirements. This information might be supplied either through the peripheral nervous system by afferent impulses, or through the blood by chemical messengers or hormones. Although both of these methods of control are used the more important is believed to be the latter. It is also believed that this hormone is related to the changes in the gases of the blood.

The two gases concerned are oxygen and carbon dioxide, each of which exists in the blood partly in a combined and partly in an uncombined or free state, and it is natural to assume that it must be the latter portion that affects the activity of the respiratory center. This free portion creates a partial pressure, or tension, of the  $\text{CO}_2$  or  $\text{O}_2$  in the blood. On a first examination,

there is nothing to indicate which of the two free gases in the blood is likely to be the respiratory hormone, and it is accordingly necessary, in approaching the subject, to investigate the effect on respiration of each gas separately. Let us therefore consider the effect of increasing the free carbon dioxide of the blood on the activity of the respiratory center. This can be done by causing animals to inspire air containing varying percentages of carbon dioxide. The most definite results of this type were obtained some years ago by R. W. Scott<sup>1</sup> who, to avoid errors incurred by using anesthetics, employed decerebrate cats for his experiments. Such animals breathe normally for considerable periods of time and are, of course, entirely insensible to pain because of the removal of the higher centers. They react to increasing percentages in inspired air in a remarkably precise manner. The first increase in respiration is observed when the percentage of carbon dioxide in inspired air has risen to a little above one, and then, as the percentage is further raised, the respiratory volume increases in direct proportion, so that when about 5 or 6 per cent of carbon dioxide is being inspired it is 4 or 5 times greater than the normal. Without further investigation one could not of course say from this experiment that the increased breathing had been due to an increased percentage of free carbon dioxide in the blood supplying the respiratory center. It might, for example, be due to irritation of afferent nerve fibers in the respiratory passages. Direct analysis of the blood in Scott's experiment, however, showed that the free carbon dioxide was increased in proportion to the increase in breathing. Having established this fact the next question to present itself was: does this free carbon dioxide in the blood act directly on the center or indirectly, in that it brings about a change in some other property of the blood? The property to be thought of in this connection is the hydrogen-ion concentration, which, it will be remembered, is an expression of the reaction of the blood. An increased amount of free carbon dioxide would be expected to raise the hydrogen-ion concentration, because of its acidic qualities. It was accordingly necessary to see whether increased acidity of the blood actually occurred and, by the use of the colorimetric method, Scott showed that it did. We are therefore face to face with the problem: is it the free carbon dioxide as such, or the accompanying increase in hydrogen-ion concentration that is the real stimulus of the respiratory center? Evidence in favor of its being a change in hydrogen-ion concentration seems to be afforded by the observation that injection of acid intravenously does cause an increase in respiration and to a lesser degree, injection of alkali decreases it. It should be pointed out here, however—and this fact is often disregarded—that although intravenous injection of other acids than carbonic does cause hyperpnea, the results are much less definite and quantitative and, moreover, the injections are much more likely to cause serious damage, resulting very frequently in the death of the animal. We have observed, for example, that a sufficient injection of hydrochloric acid to bring about the same effect that is produced by inspiration of about 5 per cent of  $\text{CO}_2$  is invariably followed by the death of the animal. In both cases, that is both with  $\text{CO}_2$  and with mineral acid, the hydrogen-ion con-



centration of the blood is increased and the respirations are stimulated, but in the one case, namely with fixed acid, other effects are produced which are likely to have a fatal issue. Similar differences in the relative effects of carbonic acid and other acids on the respiratory center have been demonstrated by Hooker and others, who in perfused preparations of the respiratory center showed that solutions containing free carbon dioxide had a much greater stimulating influence on the center than other solutions having the same hydrogen-ion concentration. These experiments suggest that the free carbonic acid in the perfusion fluid must have a specific action of its own in stimulating the respiratory center and the final proof that this is so, is afforded by further experiments by R. W. Scott.<sup>2</sup> Quantities of alkali sufficient to produce a decided diminution in hydrogen-ion concentration of the blood, that is to say, a true alkalosis, were first of all injected into decerebrate cats. Varying percentages of carbon dioxide were then administered to these animals and it was found that the increase in breathing thus induced was very much the same as in normal animals; thus, the breathing became increased several times at percentages of 5 or 6 carbon dioxide in the inspired air although the blood *was still decidedly on the alkaline side*. In other words, there was marked stimulation of the respiratory center with the blood more alkaline than normal, but containing an excess of carbonic acid. Free  $\text{CO}_2$  in the blood must therefore have an effect on the respiratory center that is independent of the change it can bring about in the hydrogen-ion concentration of the blood. It is not, of course, to be denied that change in hydrogen-ion concentration may affect the center—the experiment already referred to in which mineral acids were injected intravenously shows conclusively that this is the case—but the point is that *free carbon dioxide, besides affecting the hydrogen-ion concentration, can also specifically stimulate the respiratory center*.

Now the question is: how does carbon dioxide do this? If we can answer this question in the case of the respiratory center, certain other physiologic effects of free carbon dioxide that are apparently independent of the change in hydrogen-ion concentration will be elucidated; for example, its effect in stimulating the movements of the isolated intestine and its effect on the heart beat. The answer to the question has recently been furnished by some most interesting experiments performed by Jacobs<sup>3</sup> of the University of Pennsylvania. By observing the behavior of certain ciliated and flagellated protozoa in solutions in which the same hydrogen-ion concentration was secured by using different acids, including carbonic acid, it was found that the reactions towards the solution containing carbonic acid were quite different from those of other acids. The main difference was that the solutions containing carbon dioxide produced much more marked changes within the cell than other acids which on the other hand acted much more on the cilia than carbonic acid. Similar experiments with tadpoles confirmed the conclusions that the carbonic acid must penetrate the cell wall much more readily than other acids do. In short then, the specific action of carbon dioxide must be related to its ready penetration of the cell membrane and the possibility has to be considered that after its penetration it develops its effect by produc-

ing within the cell a local increase in hydrogen-ion concentration. According to this interpretation, it penetrates the cell membrane as a molecule of  $\text{CO}_2$  or  $\text{H}_2\text{CO}_3$  and in the protoplasm reacts with other inorganic salts to produce free hydrogen-ions. Further evidence of the great penetrating power of the carbonic acid molecule is afforded by the fact that solutions containing free carbon dioxide, even though they are slightly alkaline towards indicators, may taste slightly acid. More recent experiments by Jacobs<sup>4</sup> throw further light on this interesting property of carbon dioxide. He has found, for example, that the petals of certain flowers which are normally of a bluish tint become decidedly pink when they are bathed in a solution of sodium bicarbonate containing some free  $\text{CO}_2$  but not enough to give it an acid reaction. The change to pink indicates that the interior of the petal has acquired an acid reaction although the outside fluid in which it is bathed is slightly alkaline. He has also constructed an interesting model demonstrating the same principle. This consists of a small bottle nearly filled with a faintly alkaline solution containing some indicator, on the top of which is a layer of xylene, the mouth of the bottle being closed by a thin membrane of celloidin. By immersing this bottle in a fluid composed of weak sodium bicarbonate containing a sufficient amount of free carbon dioxide to give  $\text{pH} > 7.4$  (therefore alkaline in reaction) it is found that acid penetrates through the xylene into the neutral solution in the bottle which it causes to become acid in reaction. The explanation is that the xylene has a specific solubility toward carbon dioxide which it therefore transfers to the neutral solution where, by chemical action with the salts in this solution free hydrogen-ion is produced.

These experiments then, show clearly that whereas the respiratory center is sensitive towards changes in hydrogen-ion concentration however produced, it is still more sensitive to changes in free carbon dioxide because through its great penetrating power this enters the cells of the respiratory center and causes local changes in H-ion.

These fundamental experiments indicate the importance of measuring the tension of carbon dioxide in the blood supplying the respiratory center in order to study the conditions of its hormone control. The method of determining the tension of a gas in such a solution as blood, is to expose the solution to a confined atmosphere which is then analyzed for the percentage of the particular gas in question. From the law which states that the percentage of gas in simple solution in a fluid will be proportional to the percentage of that gas in the atmosphere, it follows that this must be proportional to the tension. In the animal body the conditions for this measurement are fulfilled by the relationship into which the blood comes with the air in the alveoli of the lung. The percentage of carbon dioxide in the alveolar air must, in other words, be proportional to the percentage of carbon dioxide in simple solution in the blood plasma as it leaves the lung. It is for this reason that analysis of alveolar air for carbon dioxide has come to occupy so prominent a place in physiologic and clinical investigation.

From what has been said, it might be expected that in all cases where breathing has increased, there should be an increased percentage of carbon

dioxide in the alveolar air. Actual examination, however, shows that this is very seldom the case, it being usually found that in cases of hyperpnea the percentage of carbon dioxide in the alveolar air is sub-normal. How then does this fit in with our hypothesis? To explain it we must adduce that these cases of hyperpnea are dependent on stimulation of the respiratory center by other factors than free carbon dioxide and the question arises, what may these be? Several possibilities present themselves, one, that other acids than carbon dioxide have appeared in the blood, such as is known to occur in the late stages of diabetes and possible also in nephritis; second, that stimulation has occurred as a result of action by other respiratory hormones such as deficiency of oxygen in the blood, and third, that the respiratory center itself has become stimulated by afferent nervous impulses. It would take us beyond the scope of this review to consider each of these three possibilities in detail and we must confine ourselves mainly to the second, namely, that the center is stimulated by the decrease in the free oxygen of the blood.

That decrease in oxygen in the inspired air can stimulate the respiratory center is readily demonstrated by experiments both on laboratory animals and on man. Oxygen deficiency in the inspired air occurs in the case of man, for example, during life at high altitudes, in aeroplaning and sometimes in mining. All candidates for the aviation service in this and other countries were tested as to their reaction toward a gradual decrease in oxygen percentage in the inspired air and it was always found during these tests that when this percentage had been lowered to somewhere about 14 the respirations became excited. Deficiency of free oxygen in the blood plasma might also, however, be brought about in ways independent of a decrease of oxygen in the inspired air. For example, a diminished available amount of hemoglobin to take up this gas, as occurs, for example, in carbon-monoxide poisoning, or a decrease in the movement of blood through the pulmonary capillaries, as occurs in decompensated heart disease, will so reduce the  $O_2$ -carrying power that the tension soon falls in the capillaries. Barcroft<sup>5</sup> has proposed terms for the various forms of anoxemia (as general condition of oxygen deficiency in the blood is called) namely, anoxic when it is due to deficiency of oxygen in the inspired air; anemic, when due to faulty oxygen-carrying powers of the blood; and stagnant, when due to faulty circulation through the tissues. However produced, the anoxemia besides stimulating respiration produces many other effects, many of which are attributable to faulty oxygenation of nerve centers—numbness or tingling of the fingers, anesthesia, changes in visual acuity and in hearing, loss of memory, inability to think, muscular tremors, are for example frequent symptoms, especially in cases where the oxygen percentage has been lowered rapidly.

One effect of the oxygen deficiency on the respiratory center is that it causes this to act very rapidly, producing shallow, rapid breathing. This leads to an aggravation of the anoxemic condition because by its shallowness each breath does not cause so adequate a ventilation of the pulmonary alveoli. The anoxemic condition becomes, therefore, rapidly worse and as Haldane, Meakins and Priestley<sup>6</sup> have shown a vicious circle becomes established.

As a result of the increased breathing, brought about by anoxemia or in any of the other ways mentioned, the blood becomes overventilated in the alveoli with the result that the free carbon dioxide becomes excessively removed from it. It is "blown off" or "washed out" of the blood and as a result of this reduction in free acid the blood tends to become more alkaline in reaction, in other words, a condition of *alkalosis* becomes established. To compensate for this excess of alkali, there comes to be an increased excretion of alkali by the urine, which can readily be detected by titration with phenolphthalein, and at the same time the excretion of ammonia becomes markedly decreased indicating that to keep down the alkali the body is now converting as much ammonia as possible into urea, which is neutral. But the combination is not perfect, with the result that for some time the blood is decidedly alkaline in character. If the alveolar air be examined in these cases the percentage of carbon dioxide will be found below the normal. A similar condition of alkalosis may also result from hyperpnea due to any cause, either voluntarily, or because of pain, or because of incomplete anesthesia in surgical operations. It is the condition called "Acapnia" by Y. Henderson. After the hyperpnea has ceased, the alkalosis will tend to be compensated by lessened breathing, to retain  $\text{CO}_2$ , with a low alveolar— $\text{CO}_2$ .

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—J. J. R. M.



# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, APRIL, 1922

No. 7

## *ORIGINAL ARTICLES*

### THE NERVOUS CONTROL OF THE PYLORIC SPHINCTER\*

BY J. EARL THOMAS, M.D., ST. LOUIS, MO., AND HOMER WHEELON, M.D.,  
SEATTLE, WASH.

ACCORDING to the theories of the function of the pyloric sphincter which have been prevalent until recently<sup>1</sup> this structure has been regarded as a separate functional entity subject to a special set of reflexes from the gastric and duodenal mucosa. Gaskell<sup>2</sup> deduced from his theories of development that the pyloric sphincter has a special nerve supply, different from that of the stomach and duodenum. A similar inference might be drawn from the idea that the pyloric function is distinct from that of the rest of the gastric muscle.

Recent work<sup>3, 4, 5, 6, 7</sup> has tended to discredit the idea of a special reflex control of the pyloric sphincter and to establish the view that all of the muscle of the pyloric portion of the stomach, including the sphincter, is a single functional unit. It is a logical deduction from these findings that all of the muscle of the pars pylorica should respond in a similar way to stimulation of its extrinsic nerve supply. The nervous physiology of the stomach has been studied extensively, that of pyloric sphincter less so; however, the results obtained demonstrate in a striking way the unity of the musculature of the pars pylorica in its relation to the extrinsic nerves. (In fact a much greater difference is to be observed between the responses of the fundic and pyloric portions of the stomach, than between the responses of the sphincter and the rest of the pars pylorica.)

#### REVIEW OF LITERATURE

Openchowski,<sup>8</sup> who gave us the first systematic study of the nervous control of the stomach considered the vagus nerve entirely motor to the pyloric

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Received for publication, August 3, 1921.

sphincter. Katz and Winkler,<sup>9</sup> Dixon,<sup>10</sup> and others also demonstrated motor responses in the pylorus following vagus stimulation. Peristaltic waves in the pylorus, according to Langley<sup>11</sup> are excited and augmented because of the reception of impulses over the vagi. It is evident from these findings that the pyloric sphincter receives excitatory fibers over the vagi. That the same is true for the remainder of the stomach is shown by the following:

According to Dixon<sup>10</sup> vagus excitation augments automatic contractions and increases gastric tonicity. Morat<sup>12, 13</sup> reports that stimulation of the central end of the severed splanchnic nerves causes contraction of the stomach. Section of the splanchnics in the frog Patterson<sup>14</sup> and dog Carlson<sup>15</sup> results in a hypertonic stomach, i.e., increased gastric tonus, and in the dog an augmentation of hunger contractions. Such hypertonicity following splanchnic section is interpreted as an unopposed bulbar activity over the vagus. On the other hand Carlson<sup>15</sup> states that section of the vagi leaves the empty stomach, on the whole, permanently hypotonic, at least for 3 months. Cannon<sup>16, 17</sup> reports that vagus section results in a temporary loss of gastric tonus and a slowing or weakening of the peristalsis. According to Pavlov<sup>18</sup> vagus section below the heart permits of the retention of food in the stomach and a deficiency of pyloric action. Food is not easily carried out of the stomach. Kelling<sup>19</sup> regards the vagi as exciters to gastric tension, the result of such tonic states being rhythmic contraction. According to Ducceschi<sup>20</sup> only one vagus nerve is required to give the entire surface of the stomach a motor supply.

The accepted function of the vagi on the small intestine is that of excitation of tonus and contraction.<sup>2, 31, 32</sup> The same uniformity is shown in the distribution of the inhibitory fibers of the vagus. The studies of Langley<sup>11</sup> led him to draw the conclusion that "the body of the stomach and pylorus receive inhibitory as well as motor fibers from the vagus." These conclusions were drawn from observations on atropinized cats and rabbits during vagus excitation. These results were confirmed for the pyloric sphincter by Page May,<sup>21</sup> who used graphic methods. Wertheimer<sup>22</sup> observed reflex inhibition of the stomach following stimulation of the central end of one vagus nerve, the other remaining intact. Stimulation of the central stump of the vagus when both nerves were cut did not result in as great a degree of inhibition. This author also states that stimulation of the central stump of the severed sciatic nerve resulted in gastric inhibition. Morat<sup>13</sup> also observed gastric inactivity following stimulation of the central end of a cut vagus. Dixon<sup>10</sup> working on frogs showed that stimulation of the vagus inhibited gastric tonus and augmented gastric contractions or movements. Auer<sup>23</sup> states that only a slight degree of reflex inhibition occurs through the vagi in rabbits.

In like manner no difference can be noted in the reported effects on the pyloric sphincter and other gastric muscle following splanchnic stimulation. Inhibition and excitation have both been observed. Elliott<sup>24</sup> found that the pyloric sphincter of the rabbit was contracted by adrenalin as well as by splanchnic stimulation. Openchowski<sup>8</sup> found the splanchnic mainly motor to the stomach and pyloric sphincter in rabbits, less so in dogs. According to Schiff<sup>25</sup>

motor fibers reach the stomach from the splanchnics by way of the solar plexus. Morat<sup>12</sup> held a similar view. Contegean<sup>26</sup> states that stimulation of the splanchnics in amphibians causes contraction, especially of the circular fibers. Courtade<sup>27</sup> also showed tonic contractions of the circular fibers of the stomach following splanchnic stimulation. However, this author states that inhibition of the longitudinal fibers occurs at the time of the contraction of the circular fibers. Waters<sup>28</sup> observed that strong stimulation of the 3rd, 4th, and 5th nerves of the frog at their exit from the spinal cord resulted in contractions of the stomach and at times of the duodenum. Steinach<sup>29</sup> made similar observations. Dixon<sup>10</sup> found that stimulation of the 3rd, 4th, and 5th nerves, especially of the 4th, resulted in increased gastric tone and lessened contractions after a latent period of about 8 seconds.

As to inhibition, Katz and Winkler<sup>9</sup> from their studies of the nervous supply of the stomach, conclude that the splanchnics cause the pylorus to open, i.e., to lose tone. Similar results were reported by Oser.<sup>30</sup> According to Auer<sup>23</sup> complete reflex inhibition of the stomach can be obtained only when the splanchnics are intact. Morat<sup>12</sup> found that stimulation of the sciatic nerve, or other sensory paths, at times resulted in a "splanchnic" (motor) effect on the stomach, at other times in a "vagal" (inhibition) effect. Patterson<sup>14</sup> observed on the frog that section of the vagi with the splanchnics left intact resulted in a hypotonic stomach. Cannon<sup>16</sup> states that section of the vagi results in an unopposed constant inhibitory action of the splanchnics which causes permanent disturbances of the gastric motor functions. Wertheimer<sup>22</sup> and also Openchowski<sup>8</sup> demonstrated gastric inhibition following splanchnic stimulation.

From the literature cited the conclusion may be drawn that the vagi and splanchnics both carry motor and inhibitory impulses to the stomach as a whole as well as to the pyloric sphincter. However, there is no direct evidence to show whether in a given case motor or inhibitory effects are obtained simultaneously on gastric wall and sphincter or if one is inhibited when the other contracts. In view of the complex nature of the nerve supply such evidence is necessary before inferences relative to the functional relation of these parts can reasonably be drawn from studies of the nervous physiology.

In the course of an investigation involving the application of graphic methods to the study of the motility of the antrum and pyloric sphincter and first part of the duodenum, advantage was taken of the opportunity to obtain simultaneous records showing the effect of stimulating the extrinsic nerves to these structures. In general the findings described in the preceding review of literature were confirmed. In addition the relation of the motor and inhibitory responses of the antrum and pyloric sphincter to each other and to those of the duodenum was observed as well as the effect of stimulation of the extrinsic nerves on the rhythmicity of the pyloric sphincter, a function which has not hitherto been studied. So far as we can learn the method of obtaining simultaneous graphic records such as we used has not been applied to the investigation of this subject. In fact graphic records of any sort showing

the effect of stimulating the extrinsic nerves to the stomach, and especially to the pyloric sphincter are extremely rare in the literature.

#### EXPERIMENTAL METHODS

Our experiments were performed on dogs. In all 171 stimulations were done on 36 animals. Ether anesthesia was used for all operative procedures. In most cases ether was used throughout the experiment. In some cases morphine was used in addition to ether and in certain experiments morphine alone was relied upon after the operations were completed. The three methods of anesthesia gave similar results but the most satisfactory from every point of view was ether with a small dose of morphine.

The method of obtaining graphic records of the movements of the pars pylorica and duodenum has been described in detail in previous publications.<sup>5, 6, 33</sup> When the sphincter alone was to be studied a specially constructed balloon consisting of a finger cot stretched over two hard rubber

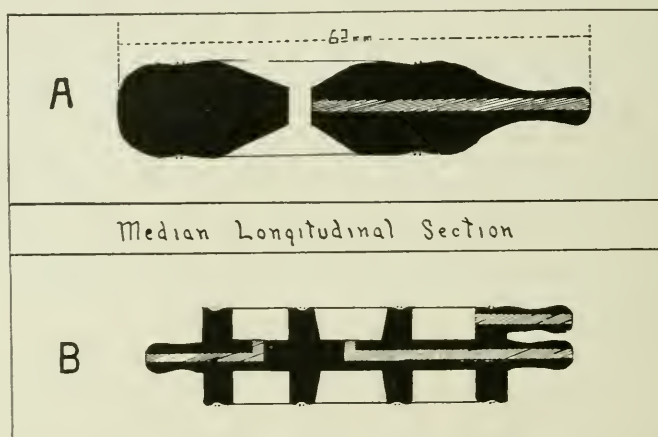


Fig. 1.—Diagram of apparatus; *A*, modified balloon or pylorograph; *B*, triple chambered balloon or triple enterogram used to record simultaneously contractions of the pyloric sphincter, antrum, and duodenum.

cones (Fig. 1*A*) was placed in the pyloric canal through an opening in the fundic end of the stomach and secured in place by sutures through the gastric and duodenal walls. When the pylorus antrum and first part of the duodenum were also to be studied a balloon divided into three parts was secured in position and a tube led from each portion so that simultaneous records could be made of antral, sphincteric, and duodenal contractions (Fig. 1*B*). Pressure was maintained in these balloons by means of a water manometer connected to each. Pressures in the chambers were varied according to our judgment of the tonus of the structures concerned. As a rule a slightly higher pressure was needed in the pyloric balloon than in the others. Pressures near 25 cms. of water were used most of the time. Registration was accomplished by means of a piston recorder for the pyloric records and bellows recorders for the other structures.

Stimulation was with the interrupted induced current from the secondary



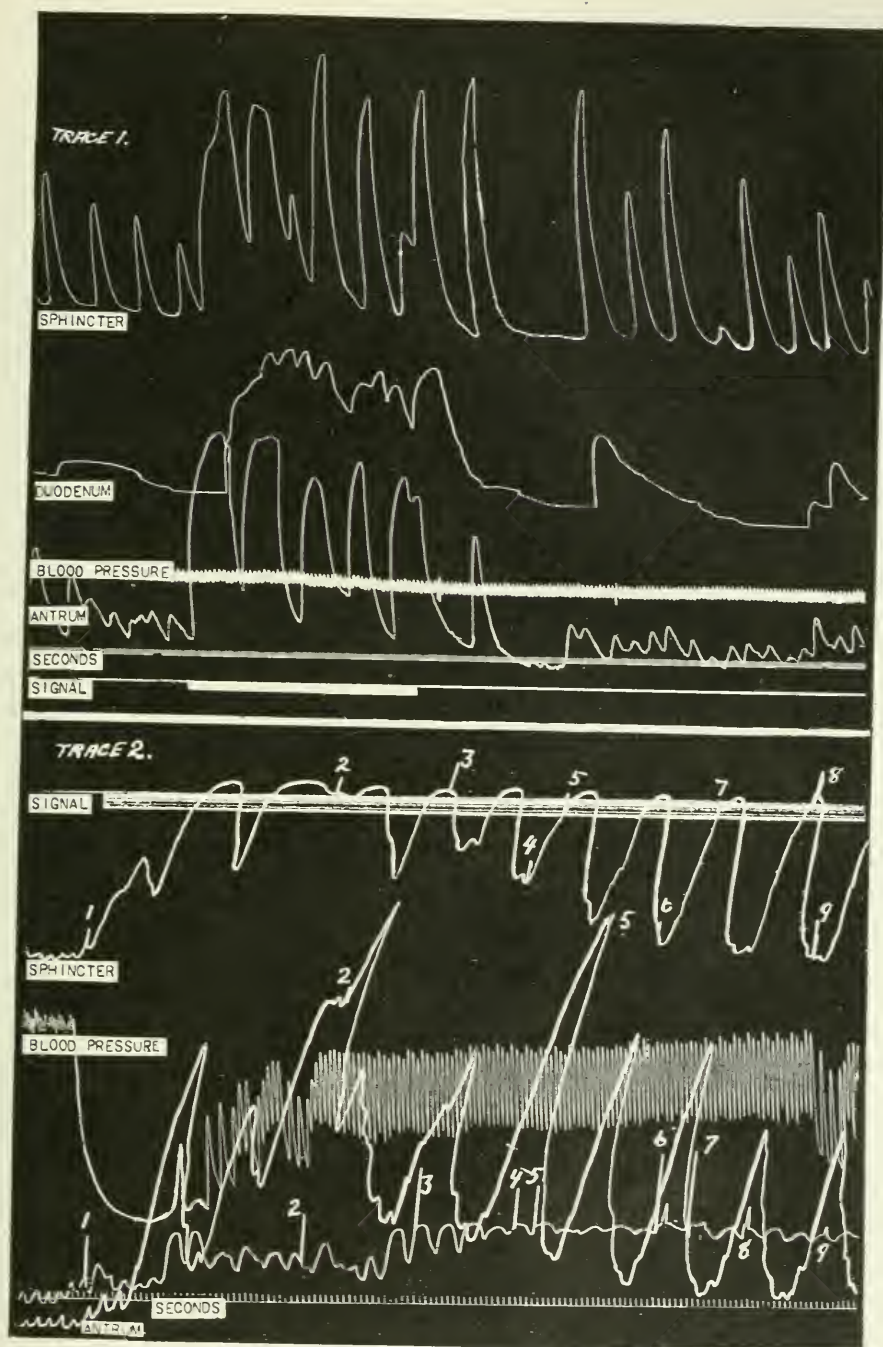


Fig. 2.—Enterograms showing the effect of vagus stimulation on the pyloric sphincter, antrum, and first part of the duodenum. Trace 1, experiment 23, February 7, 1920, ether used throughout the experiment. Vagus stimulated below the heart. Secondary coil at 9 cm. Trace 2, experiment 26, February 14, 1920, animal operated under ether, experiments conducted under the influence of sodium veronal. Vagus stimulated in the neck. Secondary of the Bois Raymond coil at 9 cm. Figures indicate synchronous points.

Note the similarity of the motor responses of the sphincter, antrum, and duodenum, also that the result is not affected by the marked circulatory disturbance, shown in trace 2.

of a Harvard or a Du Bois Raymond inductorium. The current was varied in strength according to the irritability of different preparations. In other respects it remained uniform. The primary current was obtained from a battery of storage cells connected in multiple.

In preparing the nerves for stimulation, when both the vagus and splanchnic were to be studied, a piece of one of the lower ribs was resected and both the vagi and one splanchnic, usually the left, secured and cut. The peripheral ends of the vagi were then placed in one electrode and the splanchnic in another. Electrodes of the Sherrington type were used. The thoracic incision was then closed around the wires (encased in rubber tubing), and made air-tight so that artificial respiration could be discontinued.

In many experiments we were mainly interested in the splanchnics and in these the nerves were secured below the diaphragm through a lateral incision parallel to the last rib and as near to it as possible. Frequently in these animals the vagi were picked up in the neck and stimulated. We recognize the theoretical objection to this method of vagus stimulation because of the effect it has on the circulation, nevertheless in practice the results were identical with those obtained from stimulation below the heart.

#### EXPERIMENTAL RESULTS

1. *The Effect of Cutting the Nerves.*—It was necessary to cut one or more of the nerves in preparing them for stimulation. Cannon<sup>16, 17</sup> and Carlson<sup>15</sup> have reported significant results from cutting the extrinsic nerves to the stomach. We were unable to record any constant or permanent changes in motility or tonus of the sphincter or antrum or first part of the duodenum. However, the experiments of Carlson as well as those of Cannon extended over a long period of time while our methods were adapted to record only rather abrupt changes in tonus or changes in the rhythm or force of the contractions. These did not occur under our observation and we can say with certainty that section of one or all of the nerves (vagi and splanchnics) to this region does not abolish the rhythm or seriously interfere with the tonus during the time of a single experiment under anesthesia.

2. *The Effect of Vagus Stimulation.*—Stimulation of the vagi usually produces an increase in the tonus of the pyloric sphincter. The latent period is brief, generally not longer than 5 seconds. The increase in tonus is abrupt and reaches its maximum with the first or (rarely) the second contraction; it then gradually returns to normal or below, whether or not the stimulus is continued. We have not observed a continual increase in the tonus of the sphincter with each succeeding stimulation as reported by Langley<sup>11</sup> and others (Figs. 2 and 3).

Primary decrease in tonus was observed to follow vagus stimulation with a weak current in two animals (Fig. 4). In both cases the sphincter only was under observation. Weak currents were tried in many other experiments but in these threshold currents produced primary increase in tonus. In both the experiments in which inhibition was observed it was replaced by motor effects when the strength of the current was increased. In one instance gradually

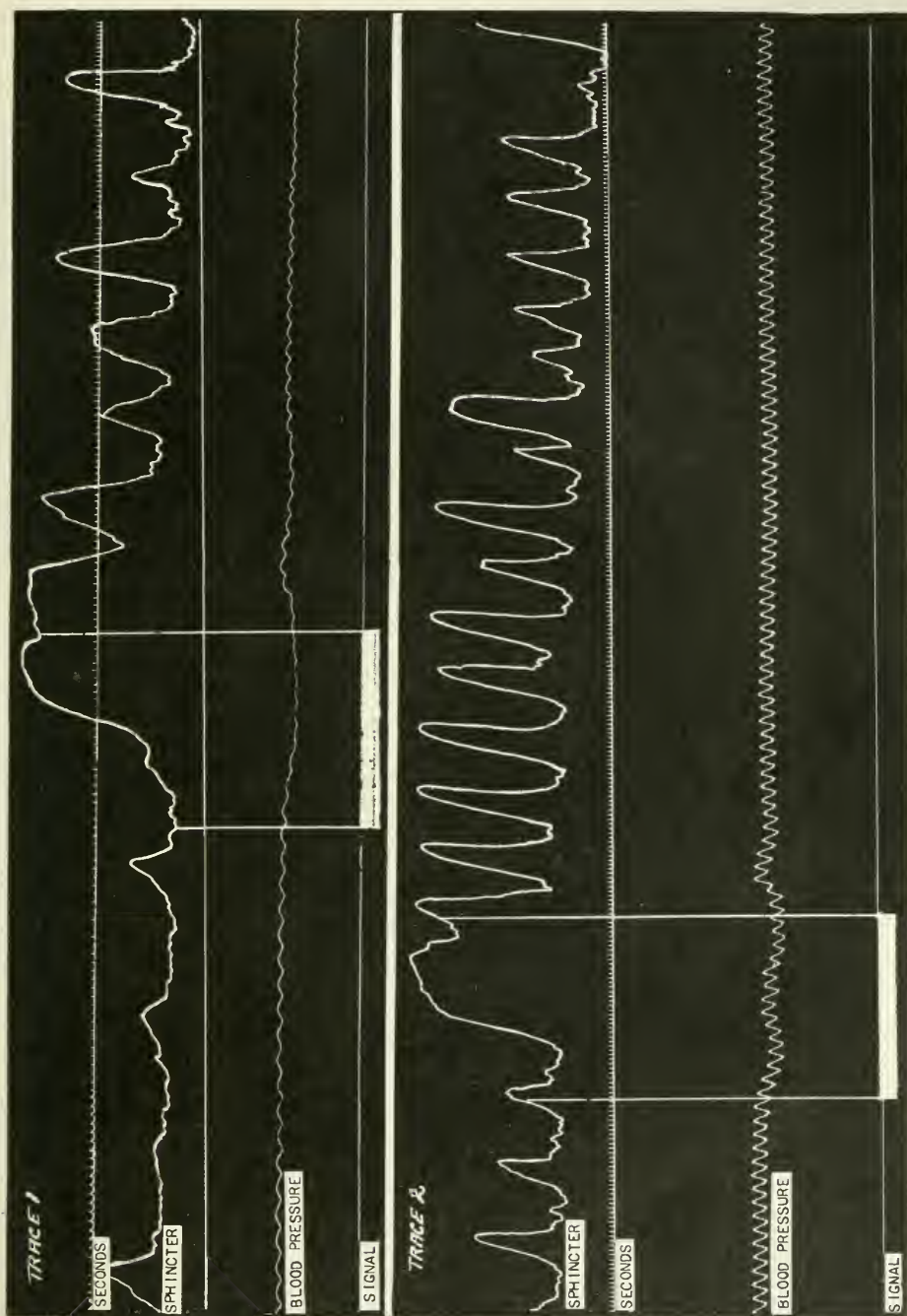


Fig. 3.—Two graphs showing the effect of vagus stimulation below the heart on the rhythm and tonus of the pyloric sphincter; trace 1, experiment 20, January 24, 1920. Ether used throughout experiment; trace 2, experiment 42, May 3, 1920, ether used throughout experiment. Secondary of Du Bois Raymond coil at 5 cm. in both cases.

Note the slight fall of blood pressure in each case, also the fact that motor responses may be obtained in a condition of great circulatory weakness. Note also the increase in the frequency of rhythmic contractions in both cases, more marked in trace 1 where the rhythm had become abnormally slow. The effect on rhythm persists after the stimulus has ceased.



increasing the strength of the stimulus diminished the inhibitory effect after it had reached a maximum. With a medium current no effect was produced. A further increase produced a motor effect which increased in extent as still stronger currents were applied.

It has recently been shown that the pyloric sphincter executes rhythmic contractions<sup>5</sup> and that these bear a definite relationship to the rhythmic activity of the pyloric antrum.<sup>6</sup> These have been observed to continue with undiminished force and normal rhythm after section of both vagi and both splanchnics, however, they are characteristically influenced by stimulation of these nerves.

During vagus stimulation the force of the rhythmic contractions increases

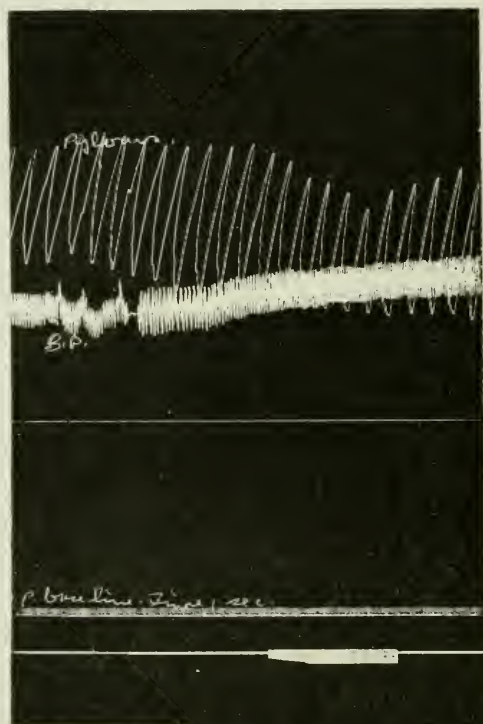


Fig. 4.—Tracing showing decrease in tonicity of the pyloric sphincter as a result of vagus stimulation. Obtained from experiment 37, March 23, 1920; secondary coil at 8 cm.; ether for operation, records obtained under morphine, 10 mm. per kg. of dog.

in nearly every case, but because of the increase in tonus and consequent lessened relaxation, the total excursion may be lessened at first. Frequently there is a spasm of the muscle which abolishes the rhythm entirely for a few seconds. At least such would be inferred from the appearance of the records. It may be well to bear in mind the limitations of the apparatus and what appears to be a spasm abolishing rhythm may very well be merely a contraction of sufficient force to obliterate the lumen of the balloon. Contraction occurring beyond this point would fail to be recorded. However, perfectly flat plateau traces such as would appear under these conditions are rare among our records.



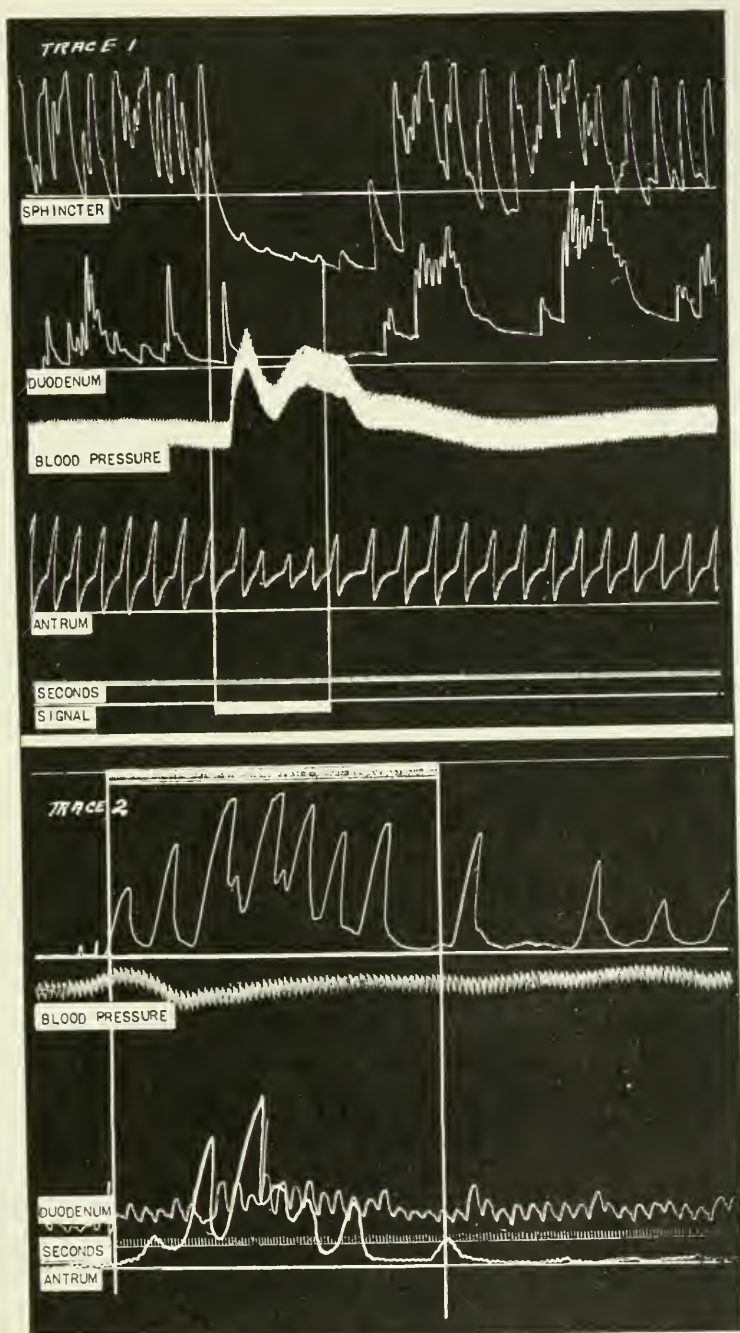


Fig. 5.—Tracings showing the effect of stimulation of the distal stump of the cut splanchnic nerve. on the motility of the pyloric sphincter, antrum, and duodenum, and the blood pressure. Trace 1, experiment 22, February 1, 1920; ether used throughout experiment; left splanchnic nerve stimulated; secondary at 8 cm. Trace 2, experiment 26, February 14, 1920; ether for operation, sodium veronal following operation. Left splanchnic stimulated, secondary at 14 cm. (Du Bois Raymond Coil.)

Note similarity of responses of sphincter, antrum, and duodenum in both cases; also that inhibition appears associated with marked vascular change, and that contraction is associated with a slight vascular effect.

On the rhythm or frequency of the contractions, stimulation of the vagi produces variable effects. A definite increase in the rate is a frequent result, especially when the rhythm has become very much slowed (Fig. 3). If the contractions have ceased they will return for a short time under effective vagus stimulation. The frequency of normal vigorous contractions remains for the most part unaltered or is only slightly increased. Slowing of the rate is not uncommon as an after effect of the stimulation; one or two contractions may be missed because of excessive tonus, or in rare cases, during a period of relaxation.

3. *The Effect of Stimulation of the Splanchnic Nerves.*—Stimulation of the splanchnics produces effects that differ a great deal, not so much in different dogs but in the same dog at different times in the experiment. We were unable to find among the factors within our control the conditions responsible for the variations. Three more or less distinct effects were recognized:

(1) Pure motor, resulting in an increase in tonus, and an increase in the strength of the rhythmic contractions of the antrum and sphincter, and frequently also of the first part of the duodenum. These effects differ only slightly from typical vagus motor effects; the chief difference is that the effect on tonus develops less abruptly, the maximum effect appearing with the third or fourth contraction instead of the first or second. Also the increase in the force of the contractions is usually less marked than after stimulation of the vagus (Fig. 5, Trace 2).

(2) Pure inhibitory, characterized by a marked loss of tonus and a complete cessation of the rhythmic contractions or a decided decrease in their force (Fig. 5, Trace 1).

(3) Mixed effects of various sorts. In many cases there was an increase in the tonus with a decrease in the force of the contractions (Fig. 6, Traces 1 and 3). Frequently the force of the contractions diminished without any apparent effect on tone. In many cases there was a primary motor effect followed by inhibition, and in others a primary inhibitory effect followed by an increase in the tonus or in the force of the contractions.

A matter of special interest in this connection is the relation of inhibitory and excitatory responses in the pars pylorica to vascular changes. As we recorded the blood pressure in practically all of our experiments we had an opportunity to observe this relationship. Our results show that in a majority of instances, motor responses in the pars pylorica from splanchnic stimulation were associated with changes in the blood pressure which were slight or in a contrary direction, that is, there was a fall instead of a rise in the blood pressure. On the other hand, in nearly every case inhibition was associated with a striking increase in the blood pressure. But the case is not quite as simple as these statements would indicate, for in certain instances (Fig. 6) a marked rise in the blood pressure is seen associated with motor responses in the gastric musculature, at least as regards tonus.

One type of mixed response which is very common and which perhaps has unusual significance in this connection develops as follows: After the usual latent period, and associated with the primary rise in the blood pressure, there

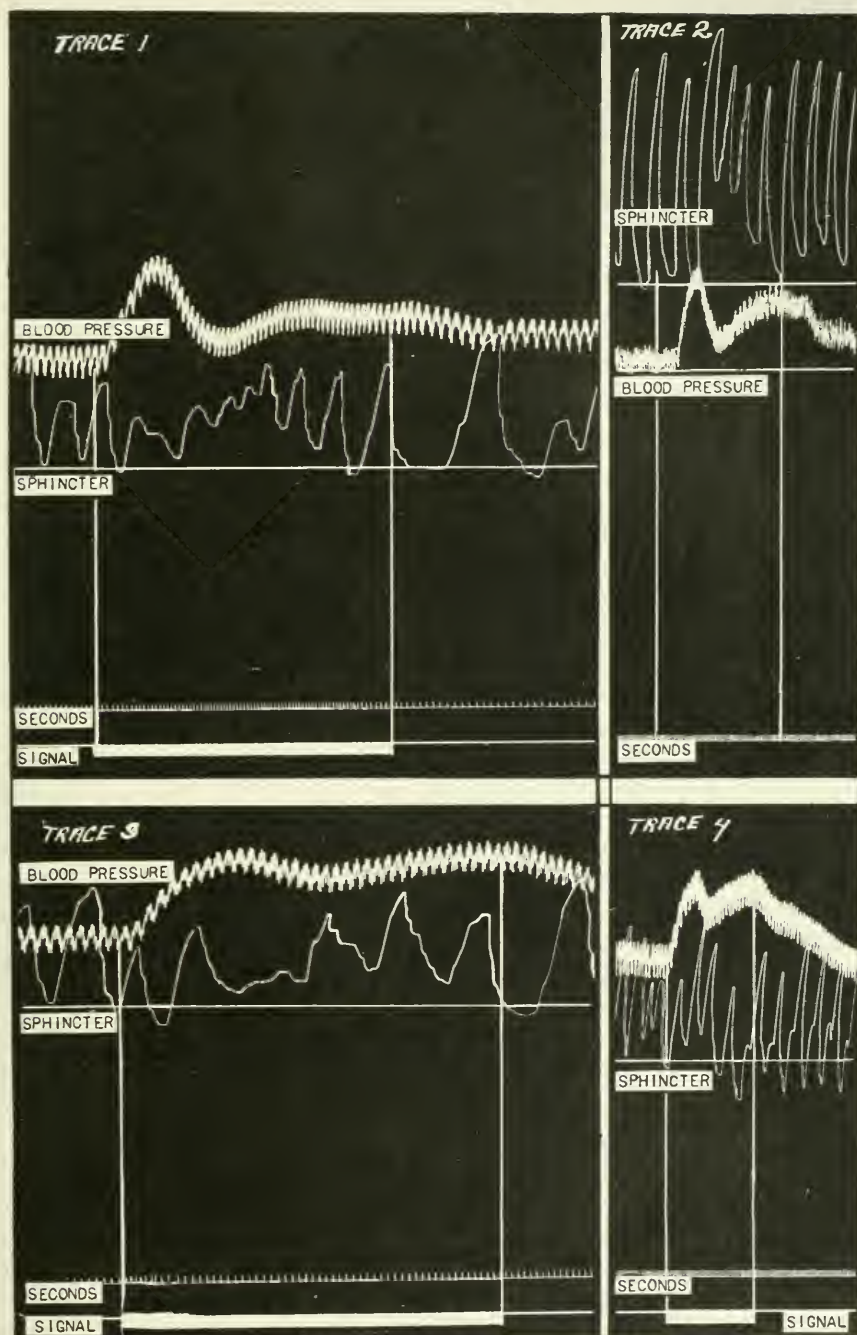


Fig. 6.—Tracings showing typical sphincter responses to splanchnic stimulation and the relation between the tonus of the sphincter and the blood pressure level during stimulation. Trace 1, experiment 19, January 22, 1920; ether used throughout experiment; right splanchnic stimulated; secondary coil at 1 cm. Trace 2, same experiment and conditions as for trace 1. Trace 3, experiment 20, January 24, 1920; left splanchnic stimulated; secondary coil at 14 cm. (Du Bois Raymond Coil.) Ether used throughout experiment. Trace 4, same as trace 3; secondary coil at 6 cm.

Note the reciprocal relation between the blood pressure level and the tonus of the sphincter.



is a slight decrease in the height of one or two contractions, but not enough to class as definite inhibition; then as the blood pressure falls, as it usually does after the primary rise, the tonus increases and the contraction which coincides with the lowest point in the blood pressure traces a curve which is higher than any other during the stimulation and frequently higher than the previous normal. As the secondary rise in the blood pressure appears the tonus falls and the strength of the individual contraction diminishes. In many cases the stimulation is followed by an increase in the tonus and the force of the contractions. These changes are shown in their most typical form in Figure 6, Trace 4, but the reciprocal relation between the tonus of the sphincter and the height of the blood pressure is apparent in all of the tracings of this figure. In Figure 5, the relation of pure motor and inhibitory reactions to the extent of the blood pressure changes is shown.

In contrast with the striking relationship seen to exist between the blood pressure changes and motor and inhibitory responses of the pylorus when these are produced by splanchnic stimulation is the absolute lack of a similar relationship when the changes are produced by vagus stimulation. As pointed out before, the motor effects following vagus stimulation are not in the least prevented by the circulatory embarrassment which accompanies stimulation of this nerve above the heart, especially in the dog. This fact is brought out in Figure 2. In Trace 2 the vagus was stimulated in the neck and the sharp drop in the blood pressure is shown. In Trace 1 the nerve was stimulated below the heart but the results are practically identical with those of Trace 2.

4. *Results Obtained when Recording Antrum, Sphincter, and Duodenum Simultaneously.*—In every instance in which simultaneous records have been obtained showing the effect of stimulating the extrinsic nerves to the antrum, sphincter, and first part of the duodenum, all of these structures have responded in the same way. Figure 2 shows two vagus motor responses affecting all three structures. Figure 5, Trace 1, shows splanchnic inhibition affecting all three simultaneously, while Trace 2 of this figure shows a splanchnic motor effect on all three at the same time. In the two instances in which we obtained inhibition over the vagus the sphincter alone was under observation so we are unable to say if the antrum and duodenum were affected in like manner.

#### SUMMARY OF EXPERIMENTAL RESULTS

In tabulating our results little attention has been given to the individual animal. The reason for this is that all the animals gave fairly uniform responses (with two exceptions) to vagus stimulation and the variations encountered on splanchnic stimulation were more often met with in the same animal at different times in the experiment than in different animals. No special variations in the technic were attempted for the purpose of varying the response and the quantitative data presented may be assumed to represent the results that may be expected from nerve stimulation with the ordinary "tetanizing" induced current in routine experiments. Table I refers to the sphincter only.



TABLE I  
SHOWING THE EFFECTS OF ELECTRICAL STIMULATION OF THE EXTRINSIC NERVES OF THE  
PYLORIC SPHINCTER

A. <i>Vagus Stimulation</i>	NO.	PER CENT	MASS ACTION	
			PER	CENT
1. Pure motor response	53	64.64	}	= 89.03
2. Primary motor, secondary inhibition	20	24.39		
3. Pure inhibition (2 animals)	5	6.09	}	= 10.96
4. Primary inhibition, secondary motor	4	4.87		
Total	82	100.00		100.00
B. <i>Splanchnic Stimulation</i>				
1. Pure Motor	29	32.58	}	= 62.91
2. Mixed, mainly motor	27	30.33		
3. Pure inhibition	28	31.48	}	= 37.09
4. Mixed, Mainly inhibition	5	5.61		
Total	89	100.00		100.00

As shown in Table I, of 82 vagus stimulations, 53, or 65 per cent, gave pure motor results; 20, or 24 per cent, gave primary motor and secondary inhibitory results; 5, or 6 per cent, gave pure inhibition, and 4, or 5 per cent, gave primary inhibition and secondary motor results.

Of 89 splanchnic stimulations 29, or 33 per cent, gave pure motor results; 27, or 30 per cent, gave mixed results which were mainly motor; 28, or 32 per cent, gave pure inhibition, and 5, or 5.6 per cent, were mixed results in which inhibition predominated.

Classifying all responses as motor or inhibitory according to which type of result predominated we have for vagus stimulation 89 per cent motor and 11 per cent inhibitory responses, a ratio of 8 motor to 1 inhibitory response. Splanchnic stimulation gave 63 per cent motor and 37 per cent inhibitory results, a ratio of 5 motor to 3 inhibitory. Of all stimulations, vagus and splanchnic, the responses were 76 per cent motor and 24 per cent inhibitory, a ratio of 3 motor to one inhibitory response. From these figures it will be seen that motor responses predominate among the results of splanchnic stimulation.

#### DISCUSSION

While this investigation was undertaken primarily to obtain more information concerning the nervous control of the pyloric sphincter, it afforded an opportunity to observe the reactions of the antrum and first part of the duodenum to stimulation of the extrinsic nerves and to compare these with the reactions of the sphincter. The fact that in every instance in which the reactions were compared they were found to be similar, may, while not conclusive alone, serve as additional evidence for assuming the functional unity of these parts. It seems at least to be opposed to the theory of Gaskell that the pyloric sphincter is a separate structure having different nervous relations from the rest of the stomach. Further, this fact makes it possible to apply the large amount of work which has been done on the nervous control of the pyloric end of the stomach to the problem of the nervous control of the pyloric sphincter and to apply the results described for the sphincter to the stomach as a whole, at least to the pyloric portion. Such an application is further justified by the

fact that our results on the sphincter are in accord with the findings of a majority of the workers who have studied the adjacent portions of the stomach. In fact the demonstration of motor and inhibitory fibers in both the vagi and splanchnics by a single method eliminates many apparent discrepancies in previous work.

One fact which seems worthy of particular emphasis is that splanchnic motor effects are distinctly not exceptional but occur with greater frequency than inhibitory results. On the other hand, while in our experiments vagus inhibitory responses are rare, they are by no means so when appropriate methods are used for their demonstration. There is a tendency to lose sight of this fact in considering the nervous physiology of the stomach. It is not uncommon in recent clinical literature as well as in text books of physiology to find the vagus referred to as the motor nerve to the stomach and the splanchnics as inhibitors. This is doubtless due to the influence of the current classification of the autonomic nerves as sympathetic and parasympathetic, a division which has never been shown to apply to the stomach. It seems entirely possible that further study will limit its application to other structures. In this connection the following personal communication from Kuntz<sup>34</sup> is of interest:

"In embryos of types of all classes of vertebrates, including man, cells of medullary and neural crest origin advance peripherally along the fibers of the motor and sensory roots respectively of the spinal nerves. Some of these cells advance into the primordia of the sympathetic trunks and prevertebral plexuses and give rise to sympathetic neurones. The neurones in the cranial sympathetic ganglia arise from cells which advance peripherally along the cranial nerves with which these ganglia are genetically associated. Likewise the neurones in the pulmonary, cardiac and enteric plexuses are derived primarily from cells which advance peripherally along the fibers of the vagus. Inasmuch as the cells which advance peripherally along the spinal and cranial nerves respectively are homologous in origin, and there are no other known sources of sympathetic neurones, embryologic studies afford no basis for functional classification of parts of the sympathetic nervous system."

An interesting comparison is possible of the nervous control of the three sphincters of the alimentary canal. It is now pretty definitely established that the cardiac sphincter is controlled entirely by the vagus.<sup>11</sup> The results here presented as well as the literature cited show quite conclusively that the pyloric sphincter has a double nerve supply part of which is of bulbar origin, coursing over the vagi and part of spinal origin distributed through the splanchnics. The origin and course of these nerves apparently does not determine their function. The ileocolic sphincter appears<sup>35</sup> to receive its nerve supply entirely from nerves of spinal origin. There is in this arrangement much more of a suggestion of anatomic convenience than there is of a special innervation of the sphincters based on their development as suggested by Gaskell, or of a specialization of function by autonomic nerves based on the anatomic course of the fibers.<sup>36</sup>

Throughout this discussion we have assumed that the results obtained by the electrical stimulation of nerve trunks represent the functions which are

normally mediated through these nerves when excited in a reflex manner. There seems to be no reason to doubt this assumption when changes in motility are the only effects to be observed, for example, when the vagus is stimulated below the heart. However, when motor phenomena are associated with marked changes in the circulation the question may properly be raised as to how much of the observed changes in motility is due to nerve fibers having a specific function and how much to vascular changes. May,<sup>21</sup> from his studies, reaches the conclusion that any effect produced by stimulation of the splanchnics, generally consisting in a diminution of motor activity, is probably due to the simultaneous effect on the vascular supply to the organ. A constriction of the blood vessels, in his estimation, produces an artificial anemia which in itself is sufficient to account for diminished gastric motor activity.

Superficially the results obtained on splanchnic stimulation appear to confirm the contention of May to some extent. In nearly every case a marked rise in the blood pressure was associated with inhibitory responses in the gastric musculature and when motor effects were obtained the vascular reaction was slight. While this is the rule there are some striking exceptions, Figure 6, Trace 2, for example. These are sufficiently frequent to discount any conclusions which may be drawn from this fact alone. Before concluding that anemia is the cause of the inhibitory responses it would also be necessary to explain why motor responses are regularly obtained on stimulation of the vagus in the neck when this causes complete stoppage of the heart for some seconds (Fig. 2, Trace 2). Certainly the failure of the circulation produced in this manner must be as complete as any caused by local vasoconstriction and yet the gastric muscle shows the same motor response as when the stimulation is accomplished without interfering with the circulation.

Obviously there is some other factor concerned in the more or less constant association of marked vascular changes and gastric inhibition when these are produced by splanchnic stimulation. In the 4 tracings shown in Figure 6 there is evident a sort of reciprocal relation between the tonus of the sphincter and the blood pressure level, but the greatest decrease in tonus does not appear until the secondary rise in the blood pressure. The secondary rise has been shown to disappear when the adrenals are ligated,<sup>38, 39, 40</sup> and is due to the liberation of adrenalin. It seems possible, therefore, that adrenalin may have something to do with the inhibitory responses obtained from splanchnic stimulation. This is, of course, not in keeping with the finding of Elliott<sup>24</sup> that the pylorus of the rabbit is contracted by adrenalin or that of Contejean<sup>26</sup> that adrenalin produces tetanic cramps in the toad's stomach. We were working with dogs, however, and it is entirely possible that there is a difference in the response of these animals to adrenalin. We have not made a thorough investigation of the effect of adrenalin on the dog's pylorus but from the few doses which we have given ranging from 0.2 to 0.5 mg. (total dose) we have seen only pronounced inhibition. If further investigation should confirm this result it would constitute at least one instance in which the effect of adrenalin is not identical with the effects of sympathetic stimulation.



## SUMMARY AND CONCLUSIONS

1. From a review of the literature and confirmatory experimental results it is shown that the influence of the extrinsic nerves on the motility of the pyloric sphincter is the same as their influence on the antrum. It is suggested that this fact is more in keeping with the view that regards these structures as a functional unit than with the idea that the pyloric sphincter is a separate functional entity having a special nerve supply and controlled by a special reflex mechanism.

2. Experimental evidence is produced showing that *the pyloric sphincter receives a double nerve supply consisting of motor and inhibitory nerves coursing by way of the vagi and splanchnics, both nerves being mainly motor in function. Inhibitory fibers are to be found in both nerves but more in the splanchnics than the vagi.* The innervation of the sphincters is compared and found to correspond to the segmental level from which the associated structures are supplied. Attention is called to the fact that the division of the autonomic nervous system into sympathetics and parasympathetics does not apply en masse to the stomach and pyloric sphincter.

3. A more or less constant relationship is shown to exist between changes in the blood pressure and changes in the motility of the pyloric sphincter when these are produced by splanchnic stimulation but not when produced by stimulation of the vagi. As a tentative explanation for this phenomenon it is suggested that the action of the inhibitory fibers in the splanchnics may be augmented by the liberation of adrenalin. Vascular changes alone are not considered sufficient to account for the results obtained.

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# BLOOD PRESSURE STUDIES IN ONE HUNDRED AND FORTY CASES OF DIABETES MELLITUS\*

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## I

### REVIEW OF THE LITERATURE

THERE has been some difference of opinion as regards the blood pressure in diabetes mellitus. Potain<sup>1</sup> found the blood pressure very high, higher even than that seen in nephritis. This view is shared by Teissier,<sup>2</sup> while conflicting views are those of Vaquez,<sup>3</sup> Hensen<sup>4</sup> and Orr<sup>5</sup> who all report cases showing hypotension. Goodman<sup>6</sup> has seen both high and low pressures, but low pressures more frequently. Elliott<sup>7</sup> has reported blood pressure studies in twenty-five cases of diabetes. There were thirteen males and twelve females in this series. Their average age was forty-five, average weight one hundred and fifty-six pounds and the average systolic pressure was 127. Of the thirteen cases under fifty the average systolic pressure was 107 and the twelve cases over fifty, the average systolic pressure was 150. He thought that the low systolic pressure was due to emaciation and brown atrophy or fatty heart.

Joslin<sup>8</sup> presents the following table showing that the systolic pressure in diabetes is practically normal.

TABLE I (FROM JOSLIN)

AGES	NO. OF CASES	AVERAGE BLOOD PRESSURE IN DIABETICS
15-20	38	121
21-25	33	122
26-30	56	121
31-35	39	120
36-40	64	125
41-45	75	139
46-50	116	143
51-55	127	154
56-60	103	154
Over 60	163	156
Total No. of cases	814	Average 139

Kalm<sup>9</sup> has recently pointed out that the blood pressure is normal or slightly subnormal in diabetes. Crummer<sup>10</sup> found a high blood pressure in diabetes. Severie<sup>11</sup> reported blood pressure findings in twenty-nine cases of diabetes. He found many ranges of blood pressure up to 240. Janeway<sup>12</sup> states that he has seen both high and low blood pressures in diabetes, the latter in severe cases with marked emaciation and diacetic acid in the urine, the former especially in the milder forms in stout elderly people, where

\*Received for publication, July 20, 1921.

TABLE II

CASE NO.	AGE	SEX	AGE AT ONSET OF THE DISEASE	BLOOD PRESSURE			REMARKS
				SYS.	DIAST.	PULSE PRES.	
1	20	F	19	120	80	40	Sugar in urine
1	21			120	80	40	Sugar in urine
2	44	F	44	140	90	50	Sugar in urine
2	45			140	90	50	Sugar free
2	46			140	90	50	Sugar free
3	55	F	44	130	90	40	Sugar in urine
3	56			130	85	45	Sugar free
4	26	F	23	100	60	40	(Died in coma, six hours later)
5	54	F	54	140	100	40	Sugar in urine
5	55			140	95	45	Sugar free
6	54	M	54	170	100	70	Patient has nephritis
7	54	F	51	210	110	100	Sugar in urine
7	55			200	110	90	Sugar in urine
8	29	F	29	130	80	50	Sugar in urine
8	30			125	80	45	Sugar free
9	59	M	58	145	90	55	Sugar in urine
9	60			145	90	55	Sugar free
10	30	F	30	130	90	40	Sugar in urine
10	31			130	90	40	Sugar free
11	37	M	37	120	80	40	Sugar in urine
11	38			120	80	40	Sugar free
12	58	F	50	120	60	60	Sugar in urine
12	60			130	70	60	Sugar free
12	62			130	70	60	Sugar free
12	63	F		135	75	65	Sugar free
12	64			140	80	60	Sugar free
13	48	M	48	140	90	50	Sugar in urine
13	49			140	90	50	Sugar free
14	60	F	60	210	140	70	Patient has nephritis
14	61			200	140	60	Sugar in urine
15	19	M	19	120	70	50	Sugar in urine
15	20			120	80	40	Sugar free
16	53	F	50	150	100	50	Sugar in urine
16	54			150	100	50	Sugar free
16	55			145	95	50	Sugar free
16	56			150	100	50	Sugar free
16	57			155	100	50	Sugar free
16	58			170	100	70	Sugar free
17	53	F	60	150	90	60	Sugar in urine
17	54			150	90	60	Sugar free
17	56			155	95	60	Sugar free
18	55	M	50	150	90	60	Died in coma next day
19	35	M	35	130	90	40	Sugar in urine
19	35			130	90	40	Sugar free
20	49	M	49	150	90	60	Sugar in urine
20	50			150	90	60	Sugar free
21	69	M	65	170	90	80	Sugar in urine
21	70			170	90	80	Sugar free
21	71			170	90	80	Sugar free
22	65	M	35	210	100	110	Sugar in urine
22	66			200	100	100	Sugar free
22	67			200	100	100	Has nephritis
23	11	F	11	90	50	40	Sugar in urine
23	12			90	50	40	Sugar free
24	8	M	7	80	45	35	Sugar in urine
24	9			80	45	35	Sugar free
25	42	M	40	120	70	50	Sugar in urine
25	43			120	70	50	Sugar free
25	44			120	70	50	Sugar free
26	51	M	48	140	90	50	Sugar in urine
26	53			145	95	50	Sugar free

TABLE II—CONT'D.

CASE NO.	AGE	SEX	AGE AT ONSET OF THE DISEASE	BLOOD PRESSURE			REMARKS
				SYS.	DIAST.	PULSE PRES.	
27	52	M	52	140	90	50	Sugar in urine
27	54			140	90	50	Sugar in urine
28	59	M	59	150	90	60	Sugar in urine
28	60			150	90	60	Sugar free
29	61	F	61	210	100	110	Sugar in urine. Has nephritis
29	63			210	100	110	Sugar free
30	12	F	10	100	60	40	Sugar in urine
30	13			100	60	40	Sugar free
31	45	M	40	140	100	40	Sugar in urine
31	46			140	100	40	Sugar in urine
32	32	F	32	120	70	50	Sugar in urine
32	33			120	70	50	Sugar free
33	62	M	58	160	100	60	Sugar in urine. Has nephritis
33	63			160	80	80	Sugar free
33	64			158	90	68	Sugar free
33	65			160	80	80	Sugar free
33	66			170	85	85	Sugar free
33	67			160	80	80	Sugar free
33	68			160	80	80	Sugar free
34	58	F	58	140	90	50	Sugar in urine
34	59			140	90	50	Sugar free
35	25	F	25	120	80	40	Sugar in urine
36	45	F	45	130	90	40	Sugar in urine
36	46			135	90	45	Sugar free
37	70	F	60	160	90	70	Sugar in urine
37	72			165	90	75	Sugar free
38	43	M	43	130	80	50	Sugar in urine
38	44			130	80	50	Sugar free
39	50	M	50	140	100	40	Sugar in urine
39	51			120	70	50	Sugar free
39	52			130	70	60	Sugar free
39	54			130	70	60	Sugar free
40	45	M	45	140	90	50	Sugar in urine
40	46			145	95	50	Sugar free
40	47			140	90	50	Sugar free
40	48			140	90	50	Sugar free
40	49			145	90	50	Sugar free
41	39	F	39	250	140	110	Sugar in urine. Has nephritis
41	40			200	130	70	Sugar free
41	41			200	120	80	Sugar free
42	54	M	54	130	70	60	Sugar in urine
42	55			130	70	60	Sugar free
42	56			135	75	60	Sugar free
43	41	M	41	130	90	40	Sugar in urine
44	42	M	42	140	90	50	Sugar in urine
44	44			140	90	50	Sugar free
45	34	F	34	140	90	50	Sugar in urine
46	60	M	50	150	90	60	Sugar in urine
47	40	F	40	130	90	40	Sugar in urine
47	45			140	90	50	Sugar in urine
47	46			145	90	55	Sugar free
48	46	F	43	150	90	60	Sugar in urine
48	47			150	90	60	Sugar free
48	48			140	85	55	Sugar free
48	49			150	90	60	Sugar in urine
48	50			150	90	60	Sugar free
49	32	M	32	130	90	40	Sugar in urine
49	33			130	90	40	Sugar free
49	34			130	90	40	Sugar free
50	47	M	44	150	80	70	Sugar in urine



TABLE II—CONT'D.

CASE NO.	AGE	SEX	AGE AT ONSET OF THE DISEASE	BLOOD PRESSURE			REMARKS
				SYS.	DIAST.	PULSE PRES.	
50	48			150	80	70	Sugar free
51	58	F	58	130	90	40	Sugar in urine
51	59			130	90	40	Sugar free
51	60			130	90	40	Sugar free
52	48	F	46	140	90	50	Sugar free
53	48	F	47	140	80	60	Sugar in urine
54	51	M	50	95	80	15	Sugar in urine
54	52			120	80	40	Sugar free. Myocard.
54	53			95	80	15	Died cardiac dilatation
55	50	F	49	140	90	50	Sugar in urine
55	51			140	90	50	Sugar free
56	54	M	52	150	90	60	Sugar in urine
56	55			150	90	60	Sugar free
57	32	M	32	130	85	45	Sugar in urine
57	33			130	90	40	Sugar free
58	39	M	39	140	90	50	Sugar in urine
59	58	M	44	160	90	70	Sugar in urine
59	59			150	90	60	Sugar free
59	60			155	90	65	Sugar free
60	60	M	58	120	70	50	Sugar in urine
60	61			130	80	50	Sugar free
61	50	F	49	140	90	50	Sugar in urine
62	50	F	46	140	80	60	Sugar in urine
62	51			140	80	60	Sugar free
62	52			150	90	60	Sugar free
62	53			150	90	60	Sugar free
62	54			150	90	60	Sugar free
62	55			150	90	60	Sugar free
62	56			155	95	60	Sugar free
63	69	M	67	160	90	70	Sugar in urine
64	44	M	44	120	80	40	Sugar in urine
64	45			130	90	40	Sugar free
65	58	F	55	150	90	60	Sugar in urine
65	59			155	95	65	Sugar free
65	60			160	100	60	Sugar free
65	61			160	90	70	Sugar free
66	57	M	57	130	90	40	Sugar in urine
66	58			135	95	45	Sugar free
67	48	M	48	150	90	60	Sugar in urine
67	49			150	95	55	Sugar free
68	57	M	50	160	85	75	Sugar in urine
69	47	M	47	140	100	40	Sugar in urine
70	54	M	51	120	80	40	Sugar in urine
70	55			120	80	40	Sugar in urine
70	56			120	80	40	Sugar free
71	36	F	35	120	80	40	Sugar in urine
71	37			120	80	40	Sugar free
71	38			120	80	40	Sugar free
72	43	M	40	120	70	50	Sugar in urine
72	44			120	70	50	Sugar free
73	45	M	45	125	75	50	Sugar free
74	68	F	66	210	80	130	Sugar in urine. Has nephritis
74	69			200	80	120	Sugar free
75	40	F	40	140	100	40	Sugar in urine
75	40			140	100	40	Sugar free
76	41	M	39	130	90	40	Sugar in urine
76	42			130	90	40	Sugar free
76	43			140	100	40	Sugar free
77	74	M	67	150	100	50	Sugar in urine
77	75			160	100	60	Sugar free
78	55	F	52	140	100	40	Sugar in urine
79	52	M	52	140	90	50	Sugar in urine

TABLE II—CONT'D.

CASE NO.	AGE	SEX	AGE AT ONSET OF THE DISEASE	BLOOD PRESSURE			REMARKS
				SYS.	DIAST.	PULSE PRES.	
79	52			140	90	50	Sugar free
80	43	F	40	130	80	50	Sugar in urine
80	44			130	80	50	Sugar free
81	58	M	58	180	100	80	Sugar in urine.
							Aortitis and nephritis
82	32	M	32	130	90	40	Sugar in urine
82	33			130	90	40	Sugar free
83	46	M	43	140	100	40	Sugar in urine
83	47			140	100	40	Sugar free
84	63	F	63	240	110	130	Sugar in urine.
							Aortitis and nephritis
84	64			220	110	110	Sugar free
85	46	M	46	140	100	40	Sugar in urine
85	46			140	100	40	Sugar free
86	68	F	68	200	110	90	Sugar in urine. Patient has nephritis
86	69			200	100	100	Sugar free
87	50	F	42	145	90	55	Sugar in urine
87	50			140	90	50	Sugar free
88	33	F	33	140	100	40	Sugar in urine
88	34			140	100	40	Sugar free
88	35			140	100	40	Sugar free
89	42	M	42	140	100	40	Sugar free
89	43			140	100	40	Sugar in urine
89	44			150	100	50	Sugar free
89	45			140	100	40	Sugar free
89	46			140	100	40	Sugar free
90	62	M	56	210	120	90	Sugar in urine.
							Patient has nephritis
90	62			210	120	90	Sugar free
91	40	M	40	150	100	50	Sugar in urine
91	41			140	100	40	Sugar free
91	42			140	100	40	Sugar free
92	55	F	52	210	100	110	Sugar in urine.
							Patient has nephritis
92	56			190	100	90	Sugar free
93	50	M	50	140	100	40	Sugar in urine
94	57	M	57	240	100	140	Sugar in urine.
							Patient has nephritis
94	58			190	100	90	Sugar free
94	59			200	110	90	Sugar free
95	30	F	30	120	80	40	Sugar in urine
95	30			120	80	40	Sugar free
96	42	F	40	130	90	40	Sugar in urine
96	43			130	90	40	Sugar free
97	25	M	24	130	90	40	Sugar in urine
97	26			130	90	40	Sugar free
97	27			130	90	40	Sugar free
97	28			130	90	40	Sugar free
98	35	M	35	100	70	30	Sugar in urine
98	36			110	70	40	Sugar free
99	7	M	6	90	60	30	Sugar in urine
99	8			90	60	30	Sugar free
100	50	F	46	180	90	90	Sugar in urine.
							Aortitis and nephritis
100	51			180	90	90	Sugar free
101	55	M	55	130	80	50	Sugar in urine
101	55			130	80	50	Sugar free
102	55	F	51	150	100	50	Sugar in urine
102	56			150	100	50	Sugar free
102	57			150	100	50	Sugar free
103	53	M	52	120	80	40	Sugar in urine
103	54			130	90	40	Sugar free

TABLE II—CONT'D.

CASE NO.	AGE	SEX	AGE AT ONSET OF THE DISEASE	BLOOD PRESSURE			REMARKS
				SYS.	DIAST.	PULSE PRES.	
104	45	F	45	150	90	60	Sugar in urine
104	46			150	90	60	Sugar free
105	62	F	62	180	80	100	Sugar in urine.
							Nephritis
105	63			200	110	90	Sugar free
105	64			220	110	110	Sugar free
106	53	F	53	190	120	70	Sugar in urine
106				190	110	80	Sugar free
107	56	F	50	150	90	60	Sugar in urine
107	56			150	90	60	Sugar free
108	58	F	53	150	90	60	Sugar in urine
108	58			150	90	60	Sugar free
109	53	F	51	160	90	70	Sugar in urine
109	53			160	90	70	Sugar free
110	28	M	28	125	70	55	Sugar in urine
110	28			120	80	40	Sugar free
111	33	M	33	110	70	40	Sugar in urine
111	34			110	70	40	Sugar free
112	38	M	38	130	90	40	Sugar in urine
112	39			130	90	40	Sugar free
113	42	F	42	130	90	40	Sugar in urine
113	43			130	90	40	Sugar free
114	47	F	40	120	80	40	Sugar in urine
114	49			130	90	40	Sugar free
115	60	M	55	120	70	50	Sugar in urine
115	61			130	80	50	Sugar free
116	60	F	50	210	110	100	Sugar in urine.
							nephritis
116	61			220	110	110	Sugar free
117	50	F	48	210	100	110	Sugar in urine.
							Nephritis and aortitis
117	51			210	110	100	Sugar free
117	52			200	100	100	Sugar free
117	53			200	100	100	Sugar free
117	54			210	110	100	Sugar free
117	55			200	110	90	Sugar free
117	56			200	100	100	Sugar free
118	53	M	33	170	100	70	Sugar in urine
118	54			190	100	90	Sugar free
119	46	M	45	150	100	50	Sugar in urine
119	47			150	90	60	Sugar free
120	62	F	52	160	90	70	Sugar in urine
121	33	M	31	140	80	60	Sugar in urine
121	35			140	90	50	Sugar free
122	39	M	39	130	90	40	Sugar in urine
123	51	F	47	110	80	30	Sugar free
124	49	M	41	120	80	40	Sugar in urine
124	50			120	80	40	Sugar free
124	51			130	90	40	Sugar free
124	52			130	90	40	Sugar free
124	53			135	90	45	Sugar free
124	54			140	100	40	Sugar free
125	56	F	56	180	100	80	Sugar in urine
125	57			190	100	90	Sugar free
126	41	M	41	150	100	50	Sugar in urine
126	42			140	100	40	Sugar free
127	45	M	45	140	80	60	Sugar in urine
127	45			140	80	60	Sugar free
128	55	M	55	220	110	110	Sugar in urine.
							Nephritis and aortitis
129	49	M	41	160	100	60	Sugar in urine
129	50			150	100	50	Sugar free
129	51			150	90	60	Sugar free

TABLE II—CONT'D.

CASE NO.	AGE	SEX	AGE AT ONSET OF THE DISEASE	BLOOD PRESSURE			REMARKS
				SYS.	DIAST.	PULSE PRES.	
130	28	M	28	130	90	40	Sugar in urine
131	59	F	59	150	90	60	Sugar in urine
132	64	F	58	150	90	60	Sugar in urine
133	52	F	52	150	80	70	Sugar in urine
133	53			160	80	80	Sugar free
133	54			150	80	70	Sugar free
133	55			160	80	80	Sugar free
133	56			160	80	80	Sugar free
133	57			150	90	60	Sugar free
134	52	F	52	180	90	90	Sugar in urine.
							Nephritis and aortitis
134	53			180	90	90	Sugar free
135	54	M	44	150	90	60	Sugar in urine
135	55			150	90	60	Sugar free
136	38	M	36	120	70	50	Sugar in urine
137	66	F	66	140	100	40	Sugar in urine
137	66			140	100	40	Sugar free
138	68	F	58	240	110	130	Sugar in urine.
							Has nephritis
139	50	F	48	220	70	150	Sugar in urine.
							Aortic insufficiency
139	50			210	50	160	Sugar free
139	51			200	50	150	Sugar in urine
139	51			200	50	150	Sugar free
140	38	M	36	140	100	40	Sugar in urine
140	39		36	140	100	40	Sugar free

chronic nephritis or arteriosclerosis existed. He believed that the disease of itself is without influence on the arterial pressure.

Ehrmann<sup>13</sup> found that an existing acidosis in diabetics produced a low blood pressure an observation which I can confirm. Coader<sup>14</sup> reports a study of the blood pressure in twenty-seven cases of diabetes. Some of his cases were associated with albuminuria and some without albuminuria.

Some have thought that high blood pressures in diabetes were due to the high blood sugar, but this does not seem to be true.<sup>15</sup>

## II

### EXPERIMENTAL

The following tables containing the results obtained in my study of the blood pressures in one hundred and forty cases of diabetes mellitus, show the following:

1. The blood pressure in uncomplicated diabetes is normal or slightly under normal.

2. In every case in this series where there was present a high blood pressure there could be demonstrated that an existing nephritis, arteriosclerosis or aortitis was present.

3. In this series twenty-two cases of diabetes were complicated by hypertension—a percentage of about sixteen.

4. The presence or absence of sugar in the urine had no effect on the blood pressure.



5. A high blood pressure in diabetes is due to a chronic nephritis, arteriosclerosis or a cardiac hypertrophy.

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# THE COLLOIDAL BENZOIN REACTION OF CEREBROSPINAL FLUID\*

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AS substitutes for the Wassermann and the Lange colloidal gold reactions as applied to cerebrospinal fluid, several tests have been proposed whose chief claims have been reliability equal to that of the above-named reactions and greater simplicity in the performance. It must be admitted that a test, which is at the same time easy to perform and reliable in its results, would have a wide field of usefulness. Of the proposed substitutes, which usually depend upon the flocculation of colloid suspensions, the mastic test has received the widest trial, without, however, results of sufficient uniformity to warrant its replacement of the more complicated procedures. The most recently proposed substitute is a flocculation reaction which makes use of a suspension of benzoïn. Guillain, Laroche, and Lechelle<sup>1</sup> claim to have obtained with it results which parallel the Wassermann reaction, precipitation of the colloidal benzoïn occurring only in certain tubes of the series in the case of syphilitic cerebrospinal fluid. They consider it more delicate than the gum mastic test and much less subject to error than the Lange colloidal gold reaction.

## TECHNIC

Only two solutions are required: a 1:10,000 solution of chemically pure sodium chloride in distilled water, and a suspension of benzoïn resin. The latter is prepared as follows: 1 gram powdered benzoïn resin is covered with 10 c.c. of absolute alcohol and allowed to stand for 48 hours. The clear liquid is then drawn off and 0.3 c.c. added gently to 20 c.c. of distilled water, then heated to 35° C. to insure a homogeneous suspension. Both solutions must be freshly prepared and the cerebrospinal fluid must contain no trace of blood.

In performing the test, 16 hemolysis tubes are set up in series and the fluids added according to the following arrangement:

Tubes:—	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Salt:	0.25	0.5	1.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
C. S. fluid	0.75	0.5	0.5													0.0
Ben- zoin:	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

The cerebrospinal fluid, as indicated, is added only to the first three Tubes. Then 1.0 c.c. is removed from Tube 3 and transferred to Tube 4, 1.0 c.c. from Tube 4 transferred to Tube 5 and so on to the fifteenth Tube, from which 1.0 c.c. is removed and discarded. Tube 16 receives no cerebrospinal fluid and serves as a control.

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Received for publication, August 8, 1921.

The authors claim for the test that cerebrospinal fluid from patients with general paresis, tabes in evolution, or any diffuse syphilitic process of the nervous system precipitates the colloidal benzoïn in the first nine Tubes of the series (sometimes the first thirteen). The flocculation always begins with the first tube of the series. This reaction in the first nine Tubes, in their experience, always paralleled the Wassermann reaction. Normal cerebrospinal fluids gave no reaction in the first five Tubes, though they admit that with a negative cerebrospinal fluid there may be precipitation in one or two Tubes of the series (Tubes 6 or 7). As a result of their work they considered flocculation in the first five Tubes characteristic and specific for syphilis and recently<sup>2</sup> have further simplified the test, as it relates to syphilis, by reducing the number of Tubes to four (the first being omitted) and a control. In an earlier communication<sup>3</sup> their results in tuberculous meningitis, using the full series of Tubes, are reported. For this condition they also claim a specific reaction, flocculation beginning at the upper limit of the syphilitic zone (Tube 6 or 7, sometimes Tube 4 or 5) and continuing to Tube 11 or on up to Tube 15.

Panzat<sup>4</sup> has reported results based on the study of fifteen fluids. He found precipitation always feeble, sometimes absent, in the first Tube. With cerebrospinal fluids giving a positive Wassermann reaction there was precipitation up to the tenth Tube. Some of his spinal fluids with a negative Wassermann reaction gave partial precipitation, but never starting before the fifth Tube. He held that his results agree with the Wassermann reaction.

Huber<sup>5</sup> confirms the value of the test in replacing the Wassermann reaction. He cites instances where the clinical history indicated syphilis and the Wassermann was uncertain, but the colloidal benzoïn was precipitated in the first five or six Tubes, that is, in the syphilitic zone.

If the results of these French observers can be confirmed, the colloidal benzoïn reaction would be a simple procedure of great practical value. We have carried out the technic of the reaction, as given in the original communication of Guillain, Laroche and Lechelle,<sup>1</sup> on all the cerebrospinal fluids received in the laboratories of the Michael Reese Hospital during the past five months. In making up the 1:10,000 salt solution we have found it more accurate to prepare it from a 10 per cent solution of chemically pure sodium chloride, making only enough for the day's tests. The alcoholic solution of benzoïn was made in quantity, the clear liquid drawn off at the end of 48 hours and preserved in a stoppered bottle. From this the benzoïn suspension was made fresh each time. Whenever obtainable the cell count of the cerebrospinal fluid is recorded. For every case we have the results of the Wassermann, Lange colloidal gold, and Ross-Jones globulin reactions. We have arranged our results in two general groups; first, the cerebrospinal fluids from clinically syphilitic patients and all those fluids giving undoubtedly positive laboratory indications of lues; and second, the cerebrospinal fluids from non-syphilitic patients, fluids which give no laboratory evidence of syphilis.

#### SYPHILITIC SPINAL FLUIDS

Of the syphilitic cerebrospinal fluids, Table I includes twelve from known syphilitic patients, where the Wassermann, Lange and globulin reactions

were all positive and the colloidal benzoin reaction could be regarded as characteristic for syphilis. Each of these showed precipitation of the benzoin suspension in the syphilitic zone comprised by the first five Tubes. Only one (105) began definitely in the first Tube; four (92, 93, 94, 120) were partly precipitated in the first Tube but not sufficiently for us to call them positive. Eight were negative in the first Tube. All but one (104) precipitated in Tube 3 and continued through the Tubes of the syphilitic zone. But none stopped with the fifth Tube, none before the ninth, and only one (113) before the thirteenth Tube. All but four continued to the thirteenth Tube. Furthermore, in a few instances precipitation occurred even in the sixteenth or control Tube, in which there was no spinal fluid at all.

In Table II are the cerebrospinal fluids where one or all the laboratory examinations certainly indicate syphilis while the colloidal benzoin reaction is negative in the first five Tubes. Two of these, (91 and 98) showed precipitation in the third Tube; two others (42 and 103) were partly precipitated in the third Tube; 103 gave partial precipitation on through the fifth, and 98 precipitated partly in the fifth Tube. None of these can be said really to begin before the sixth Tube, which according to the original article would be a negative reaction. Only one of these (103) did not continue through the thirteenth Tube; it stopped at nine. It might almost be called positive, were it not for the doubtful precipitation in Tubes 3 to 5, where it should be most marked.

TABLE I  
SYPHILIS

WASSERMANN OR LANGE REACTION POSITIVE.																BENZOIN REACTION POSITIVE					WAS- SER- MANN	LANGE CURVE
LAB. NO.	DATE	DISEASE	CELL COUNT	ROSS- JONES	BENZOIN REACTION																	
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
93	5/10	Syphilis		++	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0 +++++5555542200	
92	5/10	Syphilis		++	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0 +++++555555421	
22	2/28	Tubes		++	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+++++555555200	
105	6/3	Syphilis		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+++++5555533200	
107	6/7	Syphilis		+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+++++5555431110	
113	6/10	Syphilis		+	0	+	+	+	+	+	+	+	+	+	+	0	0	0	0	+	+++++5555443100	
114	6/10	Syphilis		+	0	0	+	+	+	+	+	+	+	+	+	+	0	0	0	+	+++++5555433300	
136	3/24	Syphilis		+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+++++1111100000	
50	3/21	Syphilis	10	+	0	0	+	+	+	+	+	+	+	+	+	+	0	0	0	+	+1122332100	
104	6/3	Syphilis		+	0	±	±	+	+	+	+	+	+	+	+	+	+	0	0	+	+++++5555533322	
94	5/10	Syphilis		++	±	±	+	+	+	+	+	+	+	+	+	+	+	±	0	+	++5555532000	
120	6/27	Syphilis		+	±	±	+	+	+	+	+	+	+	+	+	+	0	+	±	0	+++++1555555222	

In this and the succeeding tables, the symbols under Benzoïn Reaction indicate the following: 0, no change in the suspension. ±, partial flocculation; there is some precipitation but the supernatant fluid is cloudy. +, complete reaction; a heavy, white, flocculent precipitate settles to the bottom of the tube and the supernatant fluid is clear.

Table III comprises three cerebrospinal fluids where the clinical diagnosis was syphilis but where the laboratory findings were not confirmatory. The first (95), a case with a clinical diagnosis of cerebrospinal syphilis, gave negative Wassermann, Lange and globulin reactions. The cell count was only two lymphocytes. The second case (60) was under antiluetic treatment. The patient had had a chancre 25 years before. The Wassermann and Ross-



TABLE II

## SYPHILIS

WASSERMANN OR LANGE REACTION POSITIVE. BENZOIN REACTION NEGATIVE																						
LAB. NO.	DATE	DISEASE	CELL COUNT	ROSS- JONES	BENZOIN REACTION																WASS- ERMANN	LANGE CURVE
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
42	4/1	Syphilis	3	0	0	0	±	0	0	+	+	+	+	+	+	+	+	+	0	++++	1112220000	
98	5/28	Syphilis	2	+	0	0	+	0	±	0	+	+	+	+	+	+	0	0	0	0	0	5554210000
103	6/3	Syphilis		+	0	0	±	±	±	±	+	+	+	0	0	0	0	0	0	0	++++	5555555433
111	6/10	Tabs Cerebral	99	+	0	0	0	0	0	0	0	+	+	+	+	+	+	+	0	0	++++	0012200000
110	6/10	Hemorrhage	35	+	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	0	++++	0011211000
91	5/10	Syphilis		+	0	0	+	0	0	+	+	+	+	+	+	+	+	+	+	0	++++	5555532111

Jones tests were negative but the Lange curve could indicate cerebrospinal lues. The third (108) had syphilitic symptoms clinically, and the blood Wassermann reaction was three-plus positive. The cell count was normal, the spinal fluid Wassermann reaction was negative, the Lange curve changed no more than with some normal fluids. From Huber's observations the benzoïn test should be most valuable in just such cases, but in none of the three did precipitation start before the sixth Tube. In all, precipitation continued through the thirteenth Tube and in two even through the sixteenth.

TABLE III

## SYPHILIS

CLINICALLY SYPHILIS. WASSERMANN REACTION NEGATIVE. BENZOIN REACTION NEGATIVE																						
LAB. NO.	DATE	DISEASE	CELL ROSS-COUNT JONES		BENZOIN REACTION																WASSER-MANN	LANGE CURVE
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
95	5/25	C. S. Lues	2	0	0	0	0	0	0	+	+	+	+	0	+	+	+	+	0	0	0	0
60	2/25	C. S. Lues		0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0
108	6/7	Lues	3	0	0	0	0	0	0	±	±	0	+	+	+	+	+	+	+	+	+	0

The last group of syphilitic cerebrospinal fluids (Table IV) are from old treated cases where the laboratory results might not be expected to be strongly positive, or from cases where syphilis clinically was suspected but not certain, so that one might expect weak positives. In such cases, the benzoïn reaction should be the decisive factor. In two cases (41, 43), where the Wassermann reaction was positive and the Lange curve slightly changed, there was precipitation beginning before the sixth Tube. Both of these partially precipitated in the first three and in the fifth Tubes. But what we record as partial could no more be included with the positives than it could be with the negatives. No clear precipitation appeared before Tube 7, except in one case (39) where the fourth tube precipitated, with nothing more till Tube 8. This, a treated case, had given a feebly positive Wassermann reaction. All but four continued through Tube 13. One case (14), a three-year-old child who died during an operation for cerebral tumor, had given a three-plus positive blood Wassermann reaction. The spinal puncture was performed after a month of mercurial treatment. The Lange curve was strongly suggestive of cerebrospinal lues and the lipoid antigen gave a

TABLE IV  
SYPHILIS

SUSPECTED SYPHILIS AND TREATED CASES																						
LAB. NO.	DATE	SYPHILIS	CELL COUNT	ROSS-JONES	BENZONIN REACTION																WASSER-MANN	LANGE CURVE
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
43	4/1	Treated Syphilis	3	0	±	±	±	0	±	±	+	+	+	+	+	+	+	+	0	+	1110000000	
41	4/1	Treated Syphilis		0	±	±	±	0	±	0	+	+	+	+	+	+	+	+	0	0	1111110000	
39	4/1	Treated Syphilis		0	0	0	0	+	0	0	0	±	+	+	+	+	+	+	0	+	1112222000	
112	6/8	Treated Syphilis	3	0	0	0	0	0	0	±	+	+	±	0	0	0	0	0	0	0	1111000000	
38	4/1	Treated Syphilis		0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	0	0	0000000000	
102		Syphilis	4		0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	2222211111	
14	2/18	Brain Tumor	7	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0123333000 (Blood Wassermann +++)	
117	6/22	Early Paresis	3	0	0	0	0	0	0	0	0	+	+	+	+	+	0	0	0	0	0000000000	

(Blood Wassermann +++)  
0000000000

one-plus positive reaction. Though the combined laboratory reports indicated syphilis, a glioma of the cerebellum was found at necropsy; there was no histologic evidence of luetic meningeal involvement. The colloidal benzoïn precipitated in Tubes 9 and 10.

From our results on these twenty-nine syphilitic spinal fluids we come to the conclusion that the precipitation of colloidal benzoïn in the first nine tubes of the series of sixteen is of no value in diagnosing syphilis for:

1. Precipitation beginning in the first five tubes of the series and continuing to the ninth and never going beyond the thirteenth, does not parallel the Wassermann or Lange reactions in unquestionably syphilitic fluids.

2. Precipitation in Tubes 1 to 9 does not confirm the clinical diagnosis of syphilis where the Wassermann and Lange test are negative or doubtful.

#### NONSYPHILITIC SPINAL FLUIDS

The nonsyphilitic fluids are arranged in groups according to diseases. Table V shows the reaction of colloidal benzoïn in parallel with the Wassermann and Lange reactions as carried out on fourteen meningitic fluids. Six cases of tuberculous meningitis showed precipitation between Tubes 6 and 14. Four out of the six continued to Tube 15. One (87) did not begin till the ninth tube. One (5) started with partial precipitation in Tube 3 and precipitated from Tubes 4 to 15. It was not different from the precipitation reaction of the syphilitic fluids in Table I. Another (11) precipitated throughout the series from 1 to 16. There seems to us to be no definite zone of precipitation with the tuberculous meningitic fluids.

Of two streptococcus meningitic fluids, one precipitated the colloidal benzoïn in Tubes 6 to 14; the other gave partial precipitation in Tubes 3 to 6, then complete through the rest of the dilutions. Six spinal fluids from cases of meningococcus meningitis varied from the second to the sixth tube in starting the precipitation and from the twelfth to the sixteenth in stopping.

TABLE V  
MENINGITIS

LAB. NO.	DATE	DISEASE	CELL COUNT	ROSS-JONES	BENZOIN REACTION																WASSER-MANN	LANGE CURVE
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
21	3/30	Tuberculous Meningitis	112	++	0	0	0	0	0	0	+	+	+	+	+	+	+	+	0	0	1122333300	
19	4/1	Tuberculous Meningitis	112	++	0	0	0	0	0	+	+	+	+	+	+	+	0	0	+	0	0000233320	
11	3/17	Tuberculous Meningitis	200	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		1111220000	
5	4/27	Tuberculous Meningitis		++	0	0	±	+	+	+	+	+	+	+	+	+	+	+	0	0	1112222000	
85	4/29	Tuberculous Meningitis	182	++	0	0	0	0	0	0	+	+	+	0	0	+	+	0	0	0	0000000000	
87	5/6	Tuberculous Meningitis		+	0	0	0	0	0	±	0	0	+	+	+	+	+	+	+	0	0001222210	
4	3/23	Streptococcus Meningitis	4700	++	0	0	0	0	0	+	+	+	+	+	+	+	+	+	0	0	+* 1111222210	
25	3/15	Streptococcus Meningitis		trace	0	0	±	±	±	±	+	+	+	+	+	+	+	+	+			
7	3/14	Meningococcus Meningitis		+	0	0	0	+	+	+	+	+	+	+	+	+	0	0	0	0	1111333221	
9	3/30	Meningococcus Meningitis		+	0	0	0	0	0	±	+	+	+	+	+	+	0	0	0	0	0000123331	
3	4/27	Meningococcus Meningitis		+	0	0	0	0	0	±	+	+	+	+	+	+	+	+	+	0	0011222455	
81	4/27	Meningococcus Meningitis		+	0	±	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0000001223	
80	4/27	Meningococcus Meningitis		++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	1555555432	
90	5/5	Meningococcus Meningitis		+	0	±	±	±	±	+	+	+	+	+	+	+	+	+	+	0	0011100000	

\*With cholesterinized antigen.

Six cerebrospinal fluids from epileptic patients (Table VI) all showed precipitation, but varied from Tubes 6 to 8 in starting and from 14 to 16 in stopping. One (88), starting with Tubes 6 and 7, precipitated every other tube to 15.

The fluids in Table VII, from five cases of encephalitis lethargica, are still more variable in precipitation. One (64) gave no precipitation; one (18) precipitated in Tubes 9 and 10; one (79) ran from 6 to 13 and one (83) from 7 to 14. One (46) precipitated Tubes 7 to 10, then alternated partial and complete precipitation on to the final Tube.

Table VIII shows the reaction with five fluids from cases of sclerosis. Number 6, a case of vascular sclerosis, precipitated Tubes 9 and 10; No. 2, a case of general arteriosclerosis, 7 to 9, with 12 partial; No. 23, multiple sclerosis, 7 to 11; No. 31, also multiple sclerosis, gave partial precipitation in Tubes 1, 2 and 7 and complete from 8 to 15; No. 37 gave continued precipitation only from 10 to 15; it was from a case of amyotrophic lateral sclerosis.

Table IX includes fifteen cases of nervous disorders and brain and cord lesions. These all gave negative Wassermann reactions and very slight change with the Lange test. Only two, a case of dementia precox (29), and a case of tetanus (32) were negative throughout. One started with the third Tube

TABLE VI

EPILEPSY

LAB. NO.	DATE	DISEASE	CELL COUNT	ROSS-JONES	BENZONIN REACTION																WASSER-MANN	LANGE CURVE
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
27	3/24	Epilepsy		+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1111000000
28	3/24	Epilepsy		+	0	0	0	0	0	0	0	±	+	+	+	+	+	+	0	0	0	1111100000
30	3/24	Epilepsy		0	0	0	0	0	0	0	0	+	+	0	+	±	±	+	0	0	0	0000000000
35	4/1	Epilepsy		0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	1111100000
88	5/4	Epilepsy		0	0	0	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	0000000000
78	4/27	Epilepsy		0	0	0	0	0	0	+	+	+	+	+	+	+	+	±	±	+	0	0000111000

TABLE VII

ENCEPHALITIS LETHARGICA

LAB. NO.	DATE	DISEASE	CELL COUNT	ROSS-JONES	BENZONIN REACTION																WASSER-MANN	LANGE CURVE
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
18	2/15			+	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0012210000
46	4/12		28	+	0	0	0	0	0	0	+	+	+	+	±	+	±	+	±	+	0	00111100000
64	4/1		7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0122100000
79	4/27		5	0	0	0	0	0	0	+	+	+	+	+	+	+	+	0	0	0	0	0000000000
83	4/27		2	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	0	0	1111100000

TABLE VIII

SCLEROSIS

LAB. NO.	DATE	DISEASE	CELL COUNT	ROSS-JONES	BENZONIN REACTION																WASSER-MANN	LANGE CURVE
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
23	3/24	Multiple Sclerosis		0	0	0	0	0	0	0	+	+	+	+	+	0	0	0	0	0	0	1111100000
31	3/24	Multiple Sclerosis		0	±	±	0	0	0	0	±	+	+	+	+	+	+	+	+	0	0	0000000000
37	3/24	Amyotrophic		+	0	0	0	+	0	0	0	+	0	+	+	+	+	+	+	0	0	0000000000
6	3/24	Arterio-sclerosis		0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0012211000
2	3/24	Arterio-sclerosis		0	0	0	0	0	0	0	+	+	+	+	0	±	0	0	0	0	0	1112220000

and two with the fifth. Almost all precipitated in more than two or three tubes.

The normal cerebrospinal fluids, those from which one would not expect positive Wassermann or Lange reactions, are grouped together in Table X. Only one gave no precipitation with colloidal benzoïn. Some showed precipitation in only one or two tubes, as the authors concede possible with a negative fluid, but most of them showed as much precipitation as some of the definitely syphilitic fluids. Number 82 was a case entered in the hospital under the diagnosis of auditory nerve deafness. The blood Wassermann was negative. The Lange was absolutely negative. The spinal fluid Wassermann was one-plus positive with cholesterinized human heart antigen and one-plus with syphilitic liver antigen in the incubator, but was negative with lipoid antigen and with syphilitic liver antigen incubated four hours in the ice box. There were no clinical symptoms of syphilis. The patient was discharged unimproved and readmitted a few weeks later. The blood Was-



sermann was again negative with all antigens. The Lange changed slightly in the first four tubes. The condition was diagnosed normal when discharged again. The cerebrospinal fluid from the second spinal puncture is given in the Table as 101. The benzoïn test was done three times on 82 and once on 101. With 82, the first time there was precipitation in Tubes 7 to 14; the second time in Tubes 8 to 15 and the third time in 3 to 10 with partial precipitation in 11 and 13 as shown in the Table. With 101 there was precipitation in

TABLE IX  
NERVOUS DISORDERS OR LESIONS OF BRAIN OR CORD

LAB. NO.	DATE	DISEASE	CELL COUNT	ROSS-JONES	BENZOIN REACTION																WASSER-MANN	LANGE CURVE		
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16				
84	4/26	Hydrocephalus	3	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	0	0000000000		
17	4/26	Hydrocephalus			0	0	0	0	0	0	±	+	+	+	+	+	+	+	+	+			+	+
86	4/17	Lead Poisoning			576	+	0	0	0	0	0	0	±	+	+	+	+	+	+	+			+	+
115	6/15	Hemiplegia	5	0	0	0	+	+	0	+	+	+	+	+	+	+	+	+	+	+	0	0000000000		
119	6/25	Cerebral Thrombosis	24	0	0	0	0	+	0	+	+	+	+	+	0	0	0	0	0	0	0	0122211000		
99	5/25	Cord tumor	0	+	0	0	0	0	0	0	0	0	+	+	0	0	+	0	0	0	0	1111000000		
20	4/ 6	Chorea		0	0	0	0	0	0	0	0	+	+	+	0	0	0	0	0	0	0	1111000000		
97	5/28	Pituitary Tumor	10	Trace	0	0	±	0	0	±	±	+	+	+	+	+	+	+	+	0	0	0011222200		
89	5/ 2	Neuritis	2		0	0	0	0	0	0	±	+	+	+	+	+	+	+	+	+	0	1111333000		
29	3/24	Dementia Precox			+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1111100000		
16	3/19	Convulsions	3	0	0	0	0	0	0	0	0	±	0	0	±	0	0	0	0	0	0	1111100000		
33	3/24	Cerebral Thrombosis		++	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	0	0012221000		
118	6/25	Poliomyelitis	44	0	0	0	0	0	+	0	+	+	+	0	±	0	0	0	0	0	0	0001210000		
15	4/ 1	Pernicious Anemia		+	0	0	0	0	0	0	±	0	0	0	0	0	0	0	0	0	0	1112000000		
32	3/24	Tetanus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000000000		

Tubes 5 to 7 only. Clinically, there was no thought of syphilis so we have put the case under normal fluids. But the one spinal fluid Wassermann, weakly-positive with two antigens, taken with the one weakly-positive Lange and the slightly increased cell count of twelve, from the laboratory standpoint were suggestive of syphilis. If the benzoïn test were valuable for diagnosing syphilis it should be of value here. The one result, shown in the Table, of precipitation from Tubes 3 to 10 in 82 might confirm our suspicion of syphilis. Unfortunately, the benzoïn reaction also gave two different reactions, precipitation beginning with Tubes 7 or 8. So in a case where it could be of assistance in a diagnosis it gave both a negative and a positive result.

In Table XI are grouped a few of those cerebrospinal fluids on which the benzoïn test was repeated. The fluid from a case of tabes (107) was repeated once. Both times precipitation occurred in exactly the same tubes. Fluid from a case of lethargic encephalitis (83) precipitated first from Tubes 7 to 16, skipping 15; the second time from 7 through 16. A syphilitic fluid

TABLE X  
CASES WITHOUT BRAIN OR CORD INVOLVEMENT

LAB. NO.	DATE	DISEASE	CELL COUNT	ROSS- JONES	BENZON REACTION																WASSER- MANN	LANGE CURVE	
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16			
101	5/9	Normal	24	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0	0	1111000000	
45	4/6	Normal		0	0	0	0	0	0	+	+	+	+	+	+	±	±	0	0	0	0	0002220000	
100	5/17	Normal		0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	1111100000	
96	5/17	Normal		0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	0	0	0	0	0000000000
106	6/6	Normal		+	0	0	0	0	0	0	+	+	+	+	+	+	+	0	0	0	0	0	0000000000
109	6/7	Normal		0	0	0	0	0	0	±	+	+	+	+	±	0	0	±	0	0	0	0	0000000000
10	3/24	Normal		0	0	0	0	0	0	0	+	+	+	+	0	0	0	0	0	0	0	0	1111100000
12		Rachitis		+	0	0	0	0	0	0	+	+	+	+	0	0	0	0	0	0	0	0	1111100000
13	4/1	Accident	Trace	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1111100000	
24	3/24	Normal	+	0	0	0	0	0	0	0	+	+	+	+	+	+	0	0	0	0	0	0000000000	
26	3/24	Normal	+	0	0	0	0	0	0	0	+	±	+	+	+	+	±	+	0	0	0	0000000000	
44	4/1	Normal	+	0	0	±	0	0	0	+	0	±	±	±	±	+	+	+	+	0	0	0000000000	
82	4/27	Normal	12	0	0	0	+	+	+	+	+	+	±	+	±	0	0	0	0	+	*	0000000000	

\*With Cholesterinized Antigen. Blood Wassermann negative.

(93) the first time precipitated Tubes 2 to 15, the second time only 5 and 6, then 9 to 14. Fluid from a patient with hemiplegia was set up three times at the same date to insure the solutions being the same. One test precipitated Tubes 7 to 16, the second precipitated Tubes 6 to 16 and the third precipitated 6 to 16 and Tubes 3 and 4 beside. A normal spinal fluid gave precipitation the first time in Tubes 7, 8 and 9 and the second time in Tubes 7 to 13. Number 92, a syphilitic cerebrospinal fluid with positive Wassermann, Lange and globulin reactions, showed precipitation, partly in the first tube and completely in the rest to 15. The second time it showed precipitation only in

TABLE XI  
REPEATED BENZON REACTIONS

LAB. NO.	DATE	DISEASE	CELL COUNT	ROSS- JONES	BENZONIN REACTION																WASSER- MANN	LANGE CURVE
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
93	5/10	Syphilis		++	±	+	+	+	+	+	+	+	+	+	+	+	+	+	0	++++	5555542200	
	5/28				0	0	±	±	+	±	±	+	+	+	+	+	+	0	0	++++	5555555421	
92	5/10	Syphilis		++	±	+	+	+	+	+	+	+	+	+	+	+	+	+	0	++++	5555555421	
	5/28				0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	++++	5555431110	
107	6/7	Tabes		+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	0	++++	5555431110	
	6/10				0	0	+	+	+	+	+	+	+	+	+	+	+	+	0	++++	5555443100	
113	6/10	Tabes		+	0	+	+	+	+	+	+	+	+	+	+	0	0	0	0	++++	5555443100	
	6/15				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++++	0012200000	
111	6/15				0	+	+	+	0	0	+	+	+	+	+	+	+	+	+	++++	0012200000	
	6/10	Tabes		+	0	0	0	0	0	0	+	+	+	+	+	+	+	0	0	++++	0012200000	
	6/15				0	0	+	±	0	0	+	+	+	+	+	+	+	+	+	++++	0012200000	
	6/15				0	0	+	0	0	+	+	+	+	+	+	+	+	0	0	++++	0012200000	
		Tuberculous																				
5	4/25	Meningitis		++	0	0	0	0	0	0	0	±	+	+	+	+	+	+	0	0	0002222000	
	4/27				0	0	0	±	+	+	+	+	+	+	+	+	+	+	+	0	0	
83	4/27	Enecephalitis		0	0	0	0	0	0	0	+	+	+	+	+	+	+	0	+	0	1111100000	
	5/6				0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	0	0	
	5/6				0	0	0	0	0	0	0	+	+	+	+	+	+	±	+	0	0	
115	6/15	Hemiplegia			0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	0	0	
	6/15				0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	0	0	
	6/15				0	0	+	0	0	+	+	+	+	+	+	+	+	+	+	0	0	
45	4/6	Normal			0	0	0	0	0	0	±	+	+	+	±	0	0	0	0	0	0	
	4/8				0	0	0	0	0	0	+	+	+	+	+	+	±	±	±	0	0	

10 and 11. The test does not seem reliable if the limits within which precipitation occurs may vary each time it is repeated.

The benzoïn reaction was also carried out on several of our cerebrospinal fluids with 1:1000 sodium chloride solution instead of 1:10,000. This technic was taken from an English abstract.<sup>6</sup> The results were not different from those where 1:10,000 sodium chloride was used. There was the same variation with fluids from similar diseases and also the same variation when the test was repeatedly performed upon one spinal fluid. In this connection attention is called to the fact that in Guillain, Laroche and Lechelle's<sup>4</sup> latest modification of the test, distilled water is used instead of salt solution. Apparently the concentration of sodium chloride, when it varies within a range from nothing to 1:1000, has no bearing upon the discrepant results obtained.

#### SUMMARY

In summing up the results of our experiments on the use of the colloidal benzoïn precipitation reaction for cerebrospinal fluids we find:

That undoubtedly syphilitic cerebrospinal fluids do not regularly precipitate in any definite zone of dilutions.

That the benzoïn reaction adds little in doubtful syphilitic cases.

That tuberculous meningitic cerebrospinal fluids do not precipitate the colloidal benzoïn in any definite range of dilutions.

That many nonsyphilitic cerebrospinal fluids do precipitate the colloidal benzoïn suspension, and even precipitate it in the so-called syphilitic zone.

That colloidal benzoïn precipitation reactions repeated on the same fluid do not give uniform results.

Our results are based on tests performed on a total of 87 cerebrospinal fluids, twenty-nine of which were diagnosed syphilitic, either clinically or from the laboratory standpoint, and fifty-eight of which were nonsyphilitic. Of the twenty-nine syphilitic spinal fluids twelve precipitated in the syphilitic zone. Each of them continued beyond that range. Of the fifty-eight nonsyphilitic spinal fluids fifteen precipitated in the syphilitic zone.

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## THE LABORATORY SPECIALIST AS A CLINICAL CONSULTANT\*

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WHEN the author was invited by our Secretary to present a paper at this meeting on laboratory work he asked what phase of the subject he should choose. He was told that although some highly technical and little used test or some new and untried laboratory procedure might possibly be more interesting to the audience, still most of the profession would get more real benefit from the paper if it contained some practical points as to how to get the most out of the laboratory examinations that are in general use today and that have been found to be of value to the practitioner.

The three points that to the author's mind seem to give the best promise of getting the most out of the laboratory examinations in general use today are: (1) to consider all laboratory findings as clinical symptoms, (2) to decide which tests you are going to make in your office laboratory and then select some laboratory to do your more time-consuming and technical examinations, (3) to call in the pathologist as a clinical consultant in the obscure cases, except where the examinations are made as a routine.

Probably the most important point of all is the first, that laboratory findings must be considered simply as additional clinical symptoms. They should only be used to assist in establishing or excluding a diagnosis and not to make a diagnosis by themselves. A single negative examination of any specimen is of no value, especially if the other clinical symptoms do not agree with it. Even a positive result must have clinical evidence to support it. The laboratory findings must check with the other clinical findings. This fault of depending too much on the laboratory findings to make a diagnosis is probably the result of the attitude of the laboratory workers as much as it is the internists. In laboratory work, as in most every other line, a little knowledge is a dangerous thing. Laboratory findings are usually so definite that the poorly prepared or unthinking laboratory worker is inclined to think that they are all in all. The more time and study given to laboratory results as clinical symptoms the more correct will be their interpretations, and the more value they will be to the profession.

The second point, that of deciding which examinations you will make in your office laboratories and which you will send away to some larger laboratory is scarcely less important than the first. Perhaps all of you do not realize that to fully equip a modern laboratory requires as much investment as to fit up a well appointed office or the operating room of a well equipped hospital. Many of the tests that we are called upon to make today, as the Wassermann reaction, the various blood chemical examinations, etc., require several hours

\*Read before the Southwest Medical Association, at Wichita, Kansas, Nov. 23, 1920.  
Received for publication, June 19, 1921.



of continuous operations so that on account of the time element alone the general practitioner should not attempt to do them. Many of the tests require special training along the lines of chemistry, bacteriology and pathology, considerably above the amount most of us possessed at the time we graduated from a medical school. The busy practitioner does not have time to acquire this special training unless he can go away for postgraduate study frequently.

There are some laboratory examinations which, on account of their nature, have to be made immediately on the specimen's being collected from the patient. Examinations of this class are the coagulation time of the blood, test for per cent of hemoglobin, darkground examinations for spirochetes in primary venereal sores, feces examinations for ameba, cell counts in spinal fluids, etc.

There is a second class of examinations which can be made at some time after the collection of the specimen, within an hour or so. This class includes red and white blood cell counts, etc.

A third class, which is probably the largest, can be examined at any time within a reasonable period after the collection of the specimen. The equipment necessary for the examination of many of these is not very elaborate. They can be examined by the physician in his office but it is usually more satisfactory to send the majority of them to some other laboratory for examination. In this class we have differential white cell count, examination of sputum for tubercle bacilli, examination of various exudates for bacteria, routine examination of urine, blood smears for malaria, etc.

A fourth class includes those examinations which require more elaborate equipment and considerable time to complete them, and which are more satisfactory if sent away to some other laboratory. This class includes the Wassermann test on blood and spinal fluid, culture work for autogenous vaccines, gold chloride test on spinal fluid for cerebrospinal infections, examination of tumors for malignancy, the tests of the newer blood chemistry, etc.

It will readily be seen that unless the physician does those laboratory examinations coming in the first group himself, or is able to have a laboratory man visit his patient, he will not be able to gain the help that can be gotten from them.

Just how many of the examinations in the other three groups he does will depend upon how much time he will be able to give and what equipment he has at his command. Much depends upon the proper equipment, such as standard solutions and stains, and these usually deteriorate on standing, even if tightly corked. The doing of the various tests frequently enough to keep one's self efficient in the technic and familiar with the findings is essential. Even one who is used to spending a large part of his time in laboratory work finds that it is necessary to keep in training, as it were, in order to be sure of his results. Therefore, it is much better to have your office equipped to do only certain tests and to send the remainder of your work to some laboratory.

It will be seen in this outline that unless the physician is able to have a laboratory man visit his patient and collect the specimen, it is necessary for

the clinician himself to be informed as to how best to collect the specimen and transport it to the laboratory so as to give the pathologist the best opportunity of gaining the information he desires. Much could be said on this point alone, but such details will have to be omitted at this time.

The doctor submitting the specimen should understand in a general way how it is to be examined, that is, he should know the principle of the test and the general steps in the technic. By this knowledge he will appreciate in just what shape the specimen should reach the laboratory. Often the most important part of the whole examination is defeated because the specimen has not been secured in the proper way or safeguarded properly while en route to the laboratory.

The last point that we wish to make, that of using the pathologist as a clinical consultant, although possibly a new idea to some, is nevertheless an important one, if we are going to get the most out of laboratory work. Perhaps some do not realize, until they stop to think, that there are today many graduate physicians who have gone into laboratory work and who have been limiting their practice to this specialty for the last five to ten years. We believe that it can be said in their favor that as a class they stick more closely to their special line than do some of the men in other fields of specialization. If a doctor hopes to get anywhere in the laboratory game today it will take his whole effort and energy and he will not have time to practice in other fields in which he is not competent.

In spite of the large amount of pay work that is done free of charge by state laboratories, thanks to the loyal support of the profession of the southwest there are many fully equipped laboratories in this section of the country, with a graduate physician in charge, limiting his practice to laboratory work. No one should try to make you believe that it requires an M.D. degree to make a Wassermann test properly. Many of our best bacteriologists are not graduate physicians. You cannot deny that some, or possibly most, recent great advances in laboratory work have been made by men outside the medical profession. It is not our desire to detract from the value of the laboratory work when done by a nurse, a technician, or a laboratory assistant. They are valuable and absolutely necessary and frequently become more skillful than many medical men who are doing this line of work. We need more of these trained assistants; yet when it comes to interpreting a laboratory result as a clinical symptom a degree in medicine and in addition several years' experience as a general practitioner, cannot help but be of some assistance.

A few instances from our experience may assist in emphasizing the points that we have outlined.

Practically every man who has done much laboratory work has repeatedly been told by some doctor when he has submitted a specimen, say of blood for a Wassermann reaction, that this patient has been to see half a dozen other doctors, has had several Wassermann tests run by different laboratories, reports from which did not always agree, and that he wants you to test it very carefully so that he can know definitely whether this patient has or has not syphilis. It cannot be done by a Wassermann test alone and it

should not be expected. Although a 4-plus positive is strong presumptive evidence of syphilis, even such a result should not be taken alone as a positive diagnosis. Many cases of syphilis do not give a positive Wassermann at the time the blood happens to be taken; this is especially true in the early primary, the tertiary stages, and in cerebrospinal lues.

You will appreciate the uselessness of a single negative examination for tubercle bacilli in sputum. The time to diagnose pulmonary or laryngeal tuberculosis is before the bacilli appear in the sputum. Of course often the patient does not present himself for examination during this incipient stage.

Throat smears or cultures for diphtheria are difficult to take, especially in young children, and a negative examination should be disregarded if the history of exposure, and especially the clinical symptoms, are at all suspicious.

Examples of similar nature might be added indefinitely but perhaps those given are sufficient to illustrate our point, that laboratory findings are always to be taken simply as clinical symptoms.

The careful pathologist should always take time to collect a brief history of the case and make a physical examination whenever the opportunity presents. With material that is sent in from the outside or through the mail by the doctor there should always be sent a summary of the history and clinical findings. This data is often of the greatest value in assisting the laboratory man in making correct "interpretations" of his results. Some may say if the laboratory man knows what the doctor is looking for it is likely to prejudice his interpretation. This should not be the case with a laboratory consultant any more than it is with a consultant in any other line of specialty. We doubtless know of doctors doing large consultation practice who never disagree with the doctor on the case and so stand in well with them. Such relations and methods may be all right from a financial standpoint, but such men are not practicing medicine.

A review of a few cases may show more clearly what we mean by this closer co-operation between the doctor and the laboratory and the use of the laboratory man as a clinical consultant.

A specimen of blood received in a vacuum bleeding tube, from Doctor M., with a request for a Wassermann and blood count. In a letter accompanying the specimen the doctor gave a history of the case stating that the patient had been losing weight slowly for a couple of years, that he appeared anemic and that there was a definite tumor palpable in the upper left abdomen, which moved with respiration. He stated that he suspected syphilis or malignant tumor. The Wassermann was made and found a 2-plus positive. I could not make a blood count as requested as most of the cells are bound up in the fibrin clot when blood is collected in a tube. Even in the face of a 2-plus positive, which I reported, I believed the case showed many symptoms of leukemia. Occasionally a weak positive Wassermann is given in these cases. I suggested that a white cell count be made. This was done and showed a leucocyte count of over 200,000, which of course completed the diagnosis.

A throat smear and culture from a suspected diphtheria case were submitted, the direct smear was negative for diphtheria as was also the culture



the next morning. On inquiry as to the symptoms of the case I was informed that the child had a temperature of 101-102° for a few days, pulse 110-115, considerable prostration and one nostril had been completely closed for several days and was discharging a slight amount of secretion. I stated that antitoxin was indicated and requested separate cultures from the nose and throat, both of which showed diphtheria positive.

A third case was one in which I was asked to go out to take blood for a Widal. As is the custom I questioned the patient as to his history and present symptoms, and made a brief physical examination. I found that he had had a fever for about a week with a few slight chills at irregular periods. His abdomen was soft and flat with no tenderness in the iliac regions but distinct tenderness and a dull aching pain in the right lumbar region. A white cell count and blood smear for differential count were taken at the same time the Widal was collected, a specimen of urine was also requested. The Widal was negative, the white blood count was 15,400 with 85 per cent polys. and the urine was loaded with pus. I reported that the laboratory findings pointed to some pus infection in the genitourinary tract and suggested that the ureters be catheterized. This was done and a large pus kidney on the right side was discovered.

In conclusion I wish to quote a point that was brought out in a recent article in the *Journal of the American Medical Association* which in my opinion was most timely. The statement was as follows: "It should be emphasized that a knowledge of the laboratory and its standards, technic, and method of reporting is more important in obtaining the information desired than the reports obtained from a number of different laboratories which may not be careful or accurate. In other words it is the clinician's duty to know the possibility of error in the laboratory he is using. It is much better to have a test repeated several times in a laboratory to whose technic one is accustomed than to have a single test made in several laboratories which will only lead to confusion."

#### DISCUSSION

*Dr. C. D. Blachly, Drumright, Okla.*—Of all the various kinds of laboratory work being done these days possibly a certain list of them can well be done by the general practitioner and should be done by him. On the other hand there is a certain list of examinations which usually can either not be done at all or only under great disadvantage.

Taking our own office as an example, since we do not try to specialize in laboratory work but only do that which can be routinely done on our patients, the first list should include red and white blood counts, and differential white blood counts, hemoglobin estimation, direct smear examination of exudates and spinal fluids, examination of blood for malarial plasmodium (more satisfactorily done on fresh unstained blood at the bedside or in office than to make blood smears and refer to distant laboratories) urinalysis, chemical and microscopical; stomach contents, chemical and microscopical; dark ground and stained smears for *Spirochete pallida* and feces examination, chemical and microscopical.

The second list which we do not try to do in our office, includes Wassermann tests on blood and spinal fluid, globulin and gold chloride on spinal fluid; tissue diagnosis of tumors, etc.; cultural examinations of throat smears for diphtheria; animal inoculations; blood chemistry, etc.

I wish to emphasize Dr. Bailey's point about the laboratory being a means of eliciting clinical symptoms and signs quite on a par with all other forms and means of diagnosis.



*Dr. E. A. Peterson, Denver, Colo.*—There has been a decided tendency on the part of some in the last year or two to discredit the findings of the laboratory and to place an overdue amount of emphasis on the clinical examination. Some probably gathered this idea from Dr. Pottenger's paper in which he stressed so vigorously the careful examination of the patient in the diagnosis of early tuberculosis. All of us who know of Dr. Pottenger's work, know that he is just as thorough and insistent on every other phase of an examination as he is on the clinical phase.

I like to regard my office as being divided into three laboratories; each of which is just as essential as any other, and without any one of which we have not given our patient the full measure of our knowledge. The physical and clinical examination of the patient I would say is done in the clinical laboratory. Then follows the x-ray examination in the x-ray laboratory and this is followed by the chemical and bacteriological analysis made in this laboratory; and I try to pass the patient through each of these laboratories in every case in which the diagnosis is at all obscure. We frequently find more satisfactory results from the clinical laboratory, whereas, in other cases, the chemical or x-ray laboratory may give us a diagnosis of tuberculosis in which the mere findings of the tuberculosis bacillus in the sputum will decide for once and for all that the patient has tuberculosis, and I want to stress here the point that so frequently the diagnosis of tuberculosis is not made in its incipency because physicians are being taught that the tubercular bacillus does not occur until a later stage. If the patient were examined, in every case of suspected tuberculosis, as soon as it is suspected, and the proper treatment carried out just as soon as the bacillus can be demonstrated, I feel that the majority of cases of tuberculosis would get well.

It has been my experience in questioning patients, that the sputum examination has usually been put off until the patient is in the second or third stage of the disease. The reason for this is that the incipient stage, to which specialists refer as not showing tubercular bacilli in the sputum, is hardly regarded as a case of sickness by the average practitioner, and I believe that the majority of cases reach the second or third stage within a few weeks after the inception of the disease. So there is no reason at the present time to hesitate to examine the sputum for tuberculosis in any suspected case, and to repeat the examination if the sickness continues and the bacilli are not found at the first examination.

*Doctor Logan Clendening, Kansas City, Missouri.*—Doctor Bailey has referred to the desirability of the clinical pathologist's practicing his specialty as a specialty. I agree to this, but find practically, at least in the community where I live, that the pathologist is usually engaged in some clinical side line, such as syphilography, or is working towards clinical practice and using clinical pathology as a stepping stone. I do not particularly blame him as the fees for doing clinical pathology compared to the fees for doing x-ray work, a sister laboratory specialty, or giving salvarsan injections are entirely too small. But I do deplore the fact. Clinical pathology of a right, ought to be an end in itself. The solution for the problem I believe to be in the employment by hospitals of clinical pathologists who will receive adequate compensation, and will be allowed to work out the problems of their specialty in the hospital's laboratory. Under these circumstances he will be called in, as a consultant, by the physicians of the hospital not only in their hospital cases but also in their general practice.

*Dr. J. H. Stokes, Rochester, Minn.*—I wish to express my appreciation of the valuable points raised by Dr. Bailey. One has only to work in association with a large group of men to appreciate the possibilities of the laboratory man as a consultant. I feel the laboratory expert is as much entitled to do his work in the light of all the findings in the case, as is the clinician. I suggest, however, that the laboratory man in his turn should be on his guard against the *ipse dixit* frame of mind which would lead him to feel that the laboratory was the court of last resort, and that his decisions have the value of absolute truth. The laboratory findings should only be regarded as part of the clinical evidence, and in the end judgment must rest upon the whole of the facts and not upon any one part. I feel that it is not out of place to emphasize that the laboratory expert should have a wholesome respect for clinical judgment particularly as to the need for certain special

examinations. I have known of laboratory men who have presumed to inform the clinical expert as to whether or not the examination called for was indicated and have even declined to perform it in the absence of any contraindication except their own opinion that it was unnecessary.

I feel that the laboratory expert is at his best in the midst of the group of clinicians with whom he works and that geographical separation of clinic and laboratory, inevitably works to some extent to the disadvantage of both.

*Dr. R. H. Needham, Fort Worth, Texas.*—It is of utmost importance that the laboratory director be a graduate physician and he should avail himself of a pathologist as a consultant, placing all clinical data before him. The pathologist should feel free to suggest and to carry out, if necessary, any laboratory work which he thinks might be of value in working out the case. One should never make the rather common mistake of consulting the pathologist and then passing over his suggestions. Teamwork is absolutely essential.

The physician should decide just what tests he wishes to perform in his office laboratory. In reaching this decision, he should be governed by his ability, equipment, time and assistance for such work. Office tests are of little value unless efficiently performed.

The more complicated tests should be sent to a larger laboratory. From my own experience as a laboratory worker and physician, I am certain that it is more profitable and satisfactory to select one laboratory for work. The doctor should try to become intimately acquainted with the laboratory staff. Also take time to discuss and view tests, technic, etc., and become familiar with the per cent of error. If more than one individual performs the Lange or Wassermann tests study and compare their work and it may surprise one how the so-called errors and discrepancies may disappear.

I do not approve of sending duplicate tests to several different laboratories. First, it is not just; second, it usually but adds to the confusion. Stick to one laboratory until convinced that the work is not up to standard. Above all, study and become familiar with the different laboratory tests and technics, in order to be able to intelligently conclude or criticize. One must know what is the standard of work in a laboratory, if the laboratory specialist is to be of value as a clinical consultant.

*Dr. Bailey (closing the discussion).*—The best arrangement is, of course, in the group practice idea where if there is a pathologist at the head of the laboratory, as there should be, he will be called in frequently as a consultant and will have access to all the other clinical evidence to assist him in making correct interpretation of his laboratory findings. If by this relationship the group is able to do more accurate and scientific work, so also can the single practitioner do better work and get the most out of the laboratory, by placing himself in close association with the laboratory.

# LABORATORY METHODS

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## A METHOD FOR PRODUCING A SPINAL DOG\*

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BY J. EARL THOMAS, M.D., ST. LOUIS, MO.

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FOR many purposes in experimental physiology and pharmacology a spinal animal furnishes the most satisfactory preparation. Methods for preparing such an animal as well as methods for decerebration of animals are described by Sollman.<sup>1</sup> References to the literature will also be found in his book. An additional method is described by Jackson.<sup>2</sup>

These methods may be classified as (*a*) cutting operations in which the cord is severed below the medulla, with or without destruction of the higher parts of the brain; (*b*) methods of producing anemia of the higher centers by ligation of the vessels or injection of inert materials which block the blood vessels; and, (*c*) elimination of the brain by injecting poisons, usually chloroform, into its circulation.

In the method to be described an effort has been made to combine the advantages of two of these methods (*b* and *c*). It consists essentially of injecting a mixture of chloroform and petrolatum into a carotid artery after ligating the other carotid and both vertebrals. When properly done this produces a spinal animal without convulsions or rigidity and apparently without shock.

In detail the method is as follows: The animal (dog) is etherized and tied down, belly up, in the usual way. The trachea is exposed and prepared for artificial respiration. Both carotids are secured and one is ligated. The ligated vessel will be available for blood pressure determination. Both vertebrals are secured and tied. The dissection for exposing the vertebrals to view, if this is thought necessary, is described by Sollman, p. 280,<sup>1</sup> and Jackson, p. 195.<sup>2</sup> Excellent drawings illustrating the dissection are shown by Jackson on the two preceding pages. With a little practice it will be found unnecessary to expose the vertebral arteries as these can be easily located with the finger passed down along the carotid sheath to the origin of that vessel. The origin of the vertebrals may be felt just a little lateral to the carotids and their identity verified by tracing them to the point where they enter the lateral processes of the cervical vertebrae. Their extensibility will permit them to be drawn up and ligated without extensive dissection.

The materials and apparatus for the injection consist of chloroform, vaseline, a 25 c.c. graduated cylinder, a large syringe with a large needle, and a means of warming the vaseline and the apparatus. For this purpose a little

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Received for publication, June 9, 1921.

water, heated in any convenient vessel is satisfactory. The vessel containing the petrolatum, and the syringe, needle, and cylinder are placed in the water to warm while the animal is being prepared for the injection.

To prepare the mixture, from 3 to 5 c.c. of chloroform are placed in the cylinder and followed by enough melted vaseline to make 20 c.c. and the mixture shaken. It is then drawn into the syringe and immediately injected into the unligated carotid. The injected mixture, which can be seen through the vessel wall, will at first be carried away from the point of injection by the circulating blood. After a certain amount has been injected the pressure of the blood will no longer be sufficient to force the mixture ahead and it will begin to back up in the carotid between the point of injection and the heart. When this occurs the carotid is clamped firmly with a hemostat proximal to

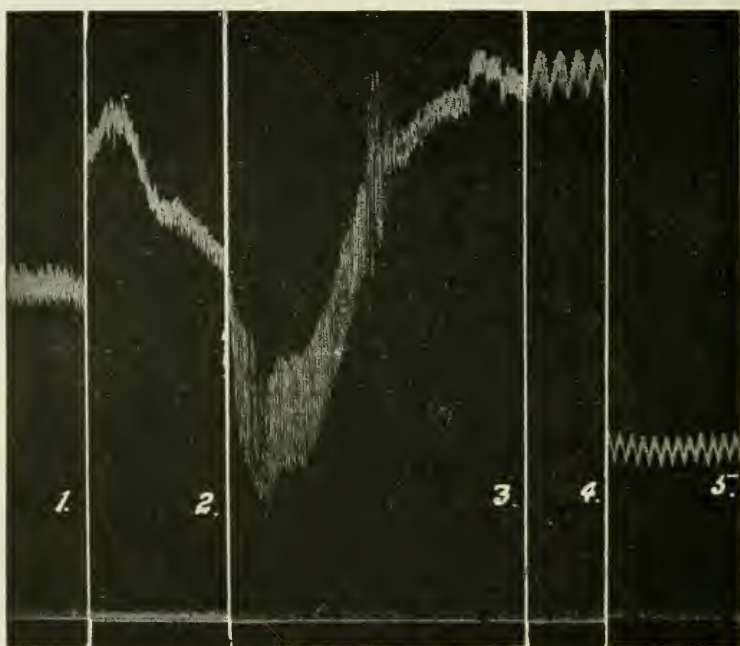


Fig. 1.—Record of the blood pressure of a 40 kilo dog during and immediately following the injection of a mixture of chloroform and vaseline into the carotid artery by the method described in the text. (1) Blood pressure under ether, before injection. (2) Rise and fall just after beginning the injection. (3) Blood pressure just before completing injection. Strong vagus action which passes off rapidly. (4) Injection completed, artificial respiration begun. (5) Blood pressure five minutes after completing the injection.

the needle and the injection continued. The respiration will cease about this time. A few c.c. should be injected after the respiration has stopped to insure against recovery. Considerable force may be safely used in completing the injection. The carotid is ligated distal to the needle before it is withdrawn to prevent escape of the mixture through the needle hole. Artificial respiration is begun whenever necessary.

During the injection the animal is likely to behave as follows: With the beginning of the injection there is a marked increase in the respiration and a considerable rise in the blood pressure. As the injection progresses this is followed by slowing and stoppage of the respiration and a fall in the blood



pressure with a very slow heart, due apparently to powerful vagus stimulation. This condition is alarming but has never proved fatal to the animal. Artificial respiration should of course be started at once. The heart may come to a complete stop but recovers promptly and the blood pressure then goes very high with no further evidence of vagus action. The condition of the heart as shown by its ability to maintain this high blood pressure as well as its recovery from the strong vagus action may be cited as evidence that chloroform does not get through to the venous circulation in dangerous amounts. The injection is usually completed soon after the vagus effect has passed off and the blood pressure then falls gradually until it reaches the level characteristic for spinal dogs. This series of events as it affects the blood pressure is shown in Figure 1. In many experiments the blood pressure has remained at nearly a normal level for an hour or longer though no evidence of a surviving medulla could be obtained. Blocking of the cerebral vessels and the consequent transference of a large amount of blood from the head to the remaining circulation may be a factor in keeping up the blood pressure.

In using this method, or any method other than actual section of the cord,

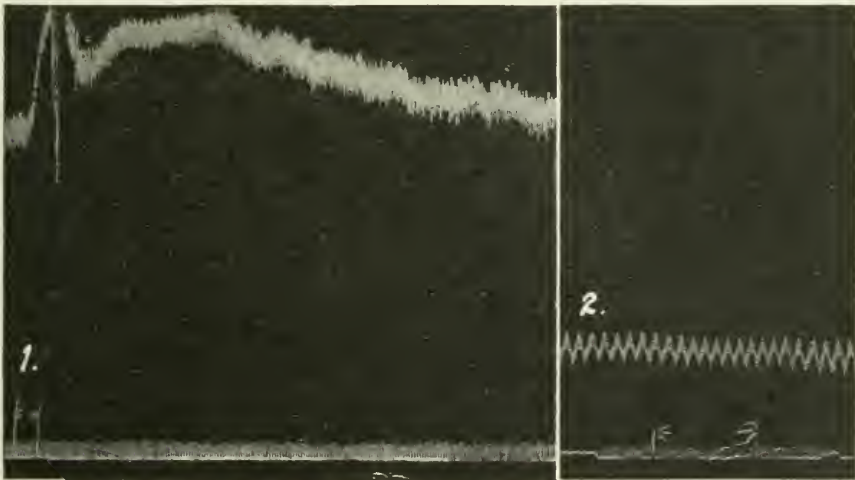


Fig. 2.—Effect on blood pressure of stimulating the central end of the left cut vagus before and after injecting chloroform-vaseline mixture into the right carotid. (1) Sharp rise in pressure with reflex slowing of the heart before injection. (2) After completing the injection. No effect.

the question will come up of whether or not the medulla is really excluded by the procedure. Several methods may be used to determine this point. All will depend on whether or not reflexes may still be obtained through the medulla. The simplest method perhaps is to observe the effect of stimulation of the central end of a cut vagus on the blood pressure. No effect will be produced in a spinal animal. Another method is to raise the blood pressure by any convenient means. If the medulla is intact there will be compensation with an evident effort to return to normal. Figures 2 and 3 illustrate the application of these methods of determining the absence of a vasoreflex mechanism. Figure 2 shows the effect of central vagus stimulation before and after injection. Figure 3 shows the degree of compensation before and after injection for a rise in blood pressure produced by equal doses of epinephrin.

The method may also be used to produce a functionally decerebrate animal by omitting the ligation of the vertebrals and stopping the injection as soon as the mixture ceases to be carried away by the blood stream, in other words, injecting at a pressure not higher than the blood pressure. For this purpose the mixture must be injected very slowly, consuming approximately five minutes in injecting the 5 or 10 c.c. that will be required for the purpose. The injection must of course be stopped and the vessel clamped if the respiration shows embarrassment. An animal so treated will usually remain quiet and live indefinitely if the temperature is artificially maintained. Death from hyperpyrexia is an occasional accident, though as a rule the temperature falls. This modification is not suitable for work in which the central nervous system is a factor because of the very obvious uncertainty as to the exact limits of

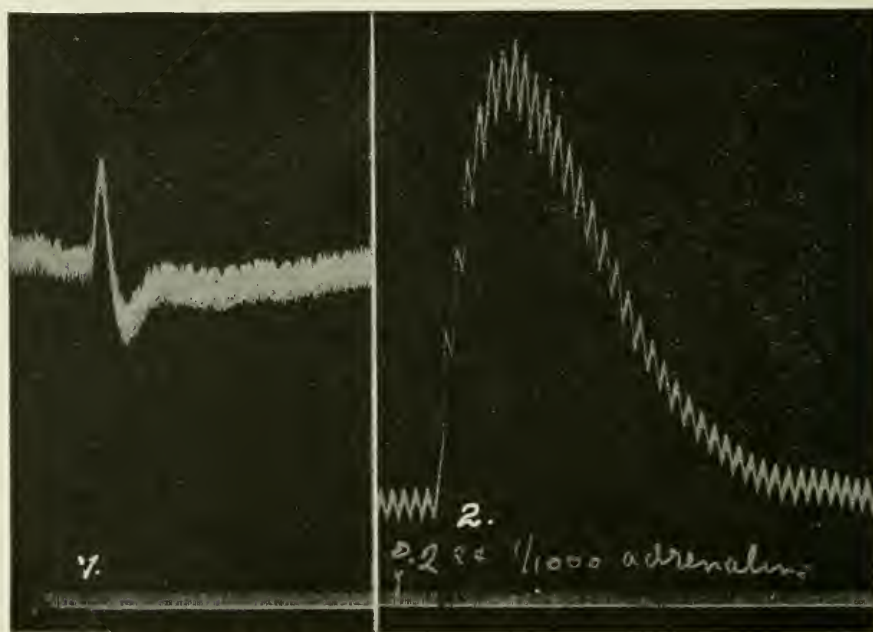


Fig. 3.—Effect on blood pressure of injecting 0.2 mg. of epinephrin intravenously (40 kilo dog) before and after destroying medulla with chloroform-vaseline mixture as described in the text. (1) Overcompensation when medulla is intact. (2) Apparent absence of compensation showing that the reflex function of the medulla has been eliminated.

the destruction produced. Means for artificial respiration should be at hand during the injection as temporary failure of the respiration is not an uncommon occurrence.

This method was devised in the Physiology Laboratory of St. Louis University School of Medicine and first used successfully in demonstrations in connection with the class work in pharmacology at that institution. It has also been used by Dr. Homer Wheelon and myself in an investigation of the pharmacology of nicotin, the results of which have not yet been published.

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- <sup>1</sup>Sollman, Thorald: A Laboratory Guide in Pharmacology, Philadelphia, 1917, W. B. Saunders Co., p. 160.
- <sup>2</sup>Jackson, Dennis E.: Experimental Pharmacology. Mosby, St. Louis, 1917, p. 195.

## A SPIROMETER FOR CONTINUOUSLY AND AUTOMATICALLY MEASURING EXPIRED AIR AND SAMPLING FOR ANALYSIS\*

BY CHARLES CLAUDE GUTHRIE, M.D., PH.D., PITTSBURGH, PA.

THE apparatus consists of two identical bell spirometers, mounted together and connected so that the expired air can be switched from one to the other without interfering with the respiration of the subject. While one is filling the other is sampled and emptied.

*Form I.*—This form has been employed for studies on dogs. The bells have a capacity of fifteen liters each. The anesthetized animal is connected by a tracheal tube with the anesthetic bottle and respiratory valves.<sup>1</sup> The expiration valve is connected by a tube to the spirometer. Room air is drawn through the anesthetic container and expelled into the spirometer automatically. The expired air is directed to one or the other spirometers by turning the tap by hand. When one is filled the tap is turned to switch the expired air to the other and the amount of air collected and time occupied, noted and recorded. A sample is taken for analysis and the bell emptied while the other is filling. With this method as with all other methods based upon the collection and analysis of expired air, when a volatile anesthetic is employed it becomes a factor in the analysis.

*Form II. Fig. 1.*—The general plan of the apparatus is the same as Form I, the essential differences being, (1) The bells are directly counterpoised, one against the other so that filling and discharging are continuous. The working capacity of each is five liters. (2) Switching the air from one to the other is entirely automatic. This is accomplished by a four way tap (Fig. 2), turned by a weight and controlled by a quadrant stop (Fig. 3). Two triggers control the stop and are worked by a double horizontal lever which in turn is moved by the bells pushing upward on the outer ends. The levers are adjusted to pull the triggers when five liters of air are introduced. (3) An indicator automatically records on a dial the total amount of air expired in five liter units, up to 180 liters and repeats without adjustment (Fig. 1, No. 13 and Fig. 4). (4) An automatic sampling device (Figs. 1, 5 and 6). This consists of an expired air chamber, an adjustable siphon and a pressure stabilizer, which at a regular rate draws one per cent, or more or less as desired, of the discharged air into a receiver for analysis. (5) Means are provided for conveniently and rapidly saturating the liquid used in the sample receiver with the subject's expired air before the sample is taken.

Taps are provided for drawing samples from either spirometer directly as in Form I.

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Received for publication, July 23, 1921.

## SAMPLE RECEIVER

This consists of a 500 c.c. narrow necked glass bottle. (Fig. 1 No. 7.) The neck is ground and fitted with a rubber stopper. The stopper is pierced with three holes. In one of these is fitted a piece of glass tubing so that the lower end is flush with the small end of the stopper. Into the other holes are fitted longer tubes which extend to near the bottom of the bottle. The lower end of one of these tubes is drawn to a narrow point and turned sharply

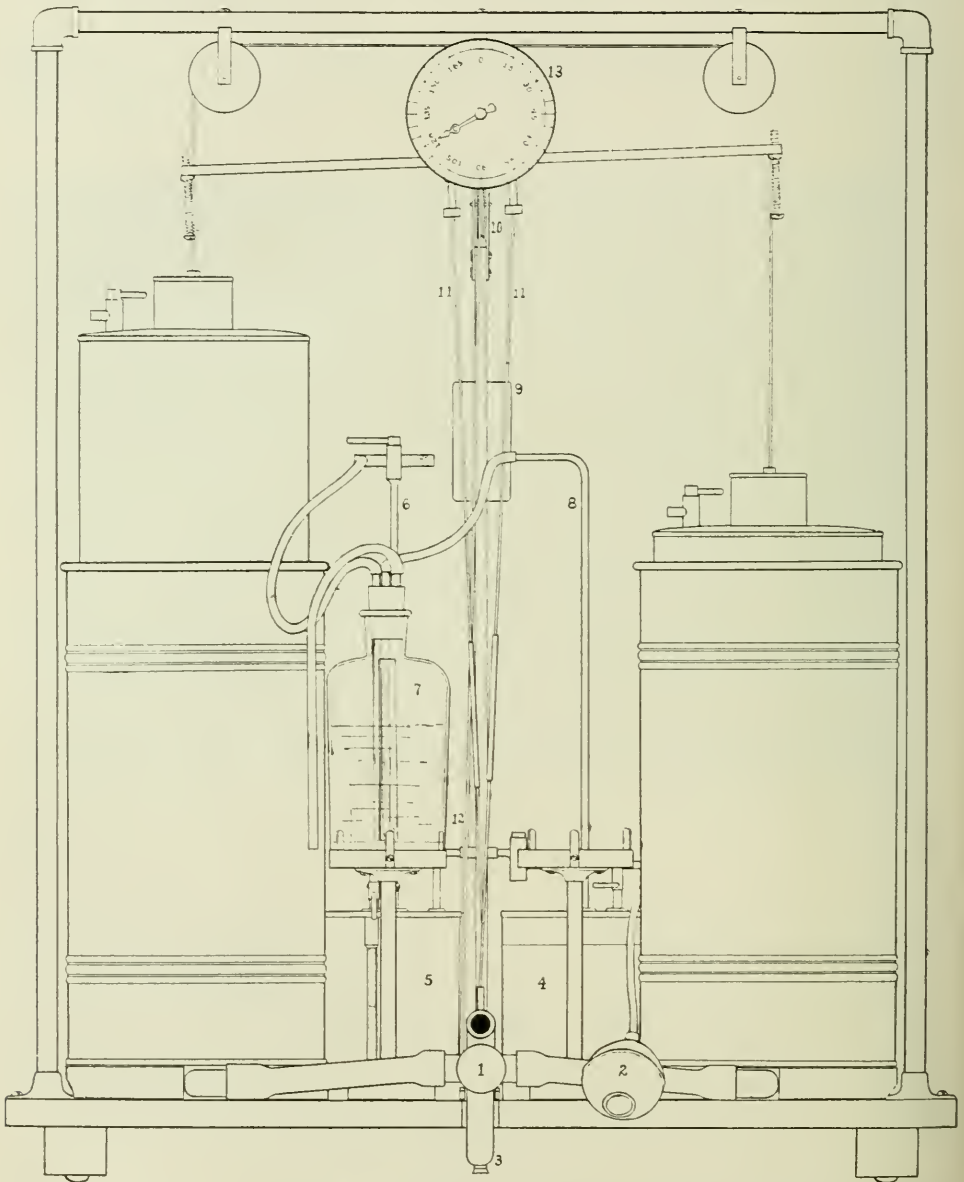


Fig. 1.—Spirometer, front view. (1) Four-way tap, with connection on shut-off tap to subject. (2) Air pump bulb. (3) Vent pipe for expired air. (4) Expired air reservoir and mixer. (5) Siphon reservoir. (6) Siphon. (7) Sample receiver. (8) Sampling tube. (9) Weight. (10) Weight pulley. (11) Trigger rods to controlling mechanism mounted behind the four-way tap. (Fig. 4.) (12) Cord from weight to driving windlass. (13) Dial.



back upon itself so the opening is directed upward. The opening is of such size that the siphon acting maximally, empties the bottle of water in about eight minutes. This tube is placed inside of a glass cylinder so that as air is drawn into the receiver, it passes to the upper part of the bottle through the cylinder, thus being limited in the extent of its contact with the mass of water in the bottle, and thus reducing loss of gases by absorption by water in case of unbalanced saturations, or partial pressures between the gases and liquid. The cylinder is of thin walled glass. The external diameter is slightly

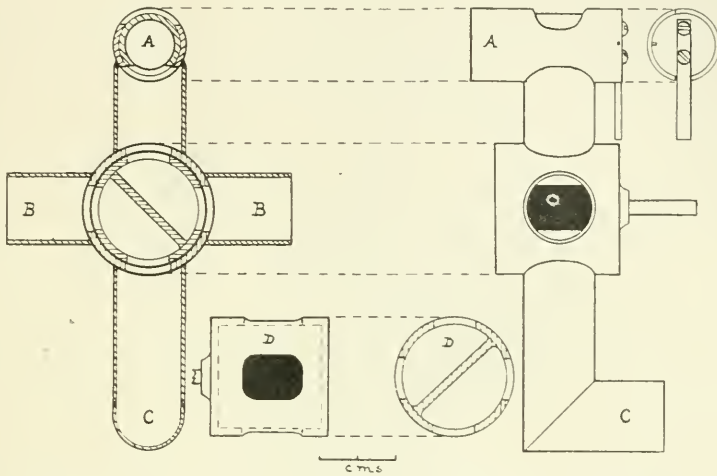


Fig. 2.—Four-way tap. *A.* Connection to subject *B.B.* Connections to spirometers. *C.* Outlet for expired air. *D.D.* Plug.

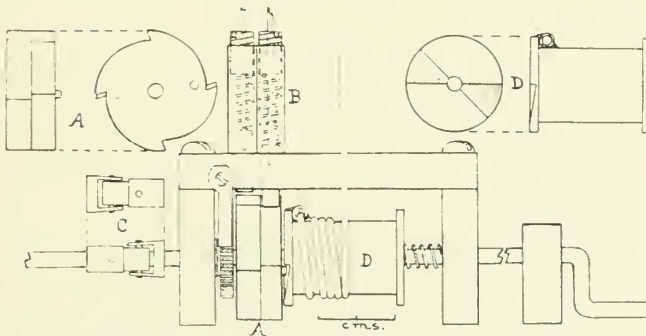


Fig. 3.—Mechanism automatically turning four-way tap. *A.* Quadrant stop. *B.* Control triggers. *C.* Drive shaft to tap, and universal joint. *D.* Driving windlass with cord to weight

less than the narrowest internal diameter of the bottle neck. The lower end rests on the bottom of the bottle. The upper end extends to within one cm. of the lower opening of the bottle neck. The outer ends of the three tubes project about a cm. above the top of the stopper and are fitted with ten inch lengths of narrow bore, pure gum tubing for making connections.

To take a sample from the spirometer, the bottle is placed upon the holder and the siphon tube connected with the siphon. The subject is connected with the spirometer and expired air is pumped through both the siphon reservoir and through the siphon which is adjusted to the top of the reservoir and then

pumped through water in the receiver, the air being permitted to escape through the short inside tube of the receiver. When the water in the reservoir and the water in the receiver is saturated with the subject's expired air the vent tube of the receiver is closed and the third or pressure tube opened. The air pressure in the reservoir is then raised by pumping into it the subject's expired air which raises the pressure in the receiver until water is forced out through the third tube. The rubber connection on this tube is then

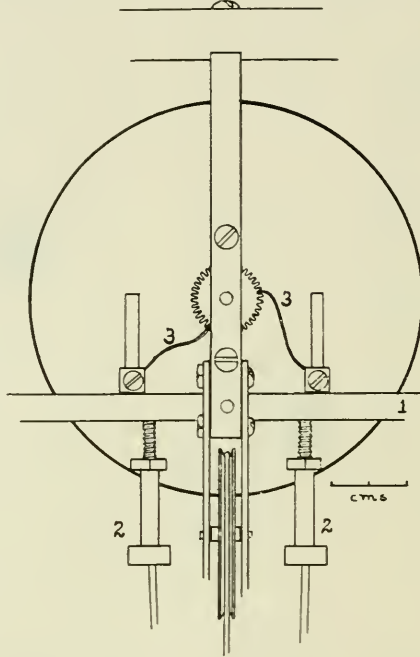


Fig. 4.—Back of dial. (1) Double horizontal lever. (2) Trigger rods. (3) Springs controlled by double horizontal lever so adjusted as to record on the dial each 5 liters of air introduced.

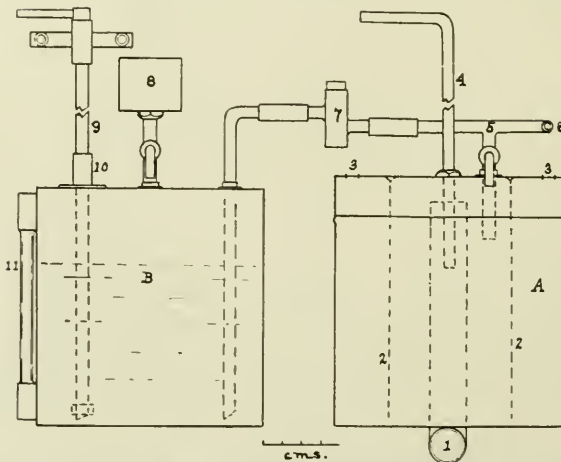


Fig. 5.—Showing details of: A, expired air reservoir and mixer; B, Siphon reservoir and connections. (1) Expiration tube from the spirometers. (2) Air baffle. (3) Vents for escape of air. (4) Sampling tube. (5) Tube for pumping expired air to siphon reservoir. (6) Pump connection. (7) Check valve on air line. (8) Filling cup and pressure equalizing vent. (9) Siphon. (10) Collar in which siphon slides. (11) Water gauge.

clamped near the end and attached to the sampling tube of the spirometer. The short inside tube or vent tube is now opened, the siphon pushed downward to the bottom of the reservoir, and the subject's expired air pumped into the reservoir, thus compressing the air and forcing the water into the re-

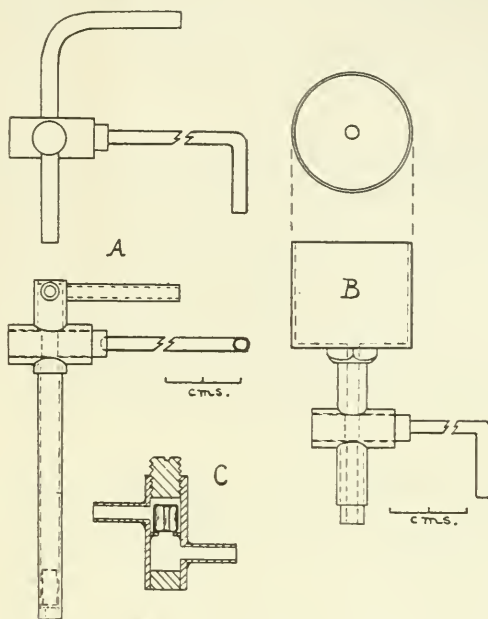


Fig. 6.—*A*, Top and side views of air connection for pumping expired air into siphon reservoir. *B*, Filling cup and pressure vent in siphon reservoir. *C*, Check valve on air line.

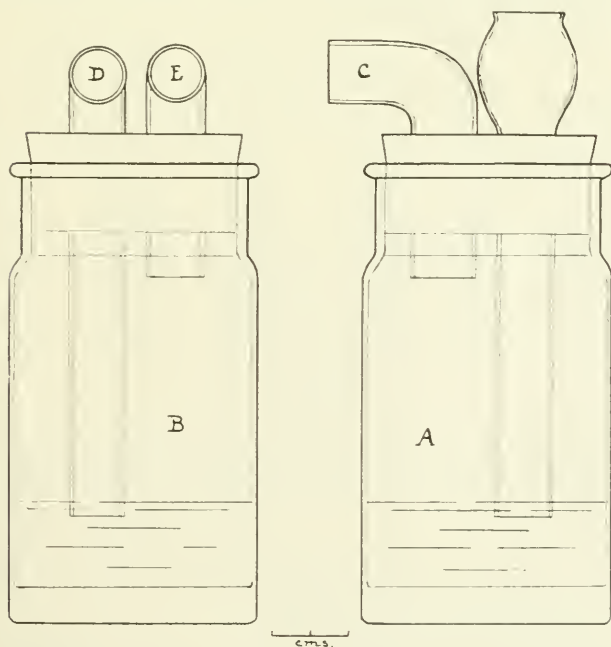


Fig. 7.—Water valves used with twin form of spirometer. *A*, Inspiration valve. *B*, Expiration valve. A rubber tube connects *C* with a *Y* to the stem of which a mouth piece is attached, and another rubber tube connects the other limb of the *Y* to *D*. *E* is connected to the spirometer tap.

ceiver. When this is completely filled with water, the vent tube is clamped near the outer end and the tap on the top of the reservoir opened to release the pressure and the siphon tube pulled upward until its lower end is checked by a stop just below the inner surface of the reservoir. The subject breathes continuously through the spirometer during this entire period which ensures that all air used for saturation purposes and that all air in the connections, i.e., the small quantity contained in the spirometer sampling tubes, is the subject's expired air.

The spirometer dial is now read for zero, the time noted and the clip on the sampling tube of the receiver removed. This starts the siphon and air is drawn from the expired air chamber of the spirometer into the receiver. When the observation is completed the time is noted, the dial read for total quantity of gas, and both the siphon and sampling tubes of the receiver are clamped near their ends and disconnected. The receiver is now ready for transferring to the holder used in connection with analyzing the sample.

To transfer samples to a gas burette the end of the siphon tube is connected with a displacing reservoir, as described elsewhere.<sup>2</sup>

#### RESULTS

Basal metabolism results for man by this form of spirometer compared to the Tissot form are shown by the following experiment. Prior to the first test the individual reclined at rest for thirty minutes.

TABLE I

Results of successive tests of oxygen consumption for ten-minute periods by a Tissot and the Twin form of spirometer. Five to ten minutes elapsed between successive tests but the subjects in each group of observations remained quiet throughout the entire period. Water valves were used in all the tests. (Fig. 7.)

DATE	STATE	FORM	TOTAL EXPIRED AIR	CARBON DIOXIDE PER CENT	OXYGEN PER CENT	RESPIRATORY QUOTIENT	BASAL RATE
1921							
April 21.	Not fasting.	Tissot	47.23 L.	4.094	15.897	0.773	+9.64
" 21.	" "	Twin	42.58	3.999	15.753	0.723	+1.87
" 21.	" "	Tissot	45.26	4.404	15.853	0.836	+6.44
" 22.	" "	Tissot	57.00	4.216	16.066	0.834	+13.89
" 22.	" "	Twin	53.72	4.025	16.183	0.812	+4.75
" 22.	" "	Tissot	52.00	4.316	15.941	0.833	+6.49
" 23.	Fasting	Tissot	44.40	4.233	15.817	0.787	-10.08
" 23.	"	Twin	42.786	4.208	15.733	0.766	-11.98
" 23.	"	Tissot	48.10	4.425	15.875	0.843	-3.71

#### SUMMARY

A form of spirometer for continuously and automatically measuring and sampling expired air is described. It was designed primarily for comparative experimental studies on laboratory animals to give results accurate to within about five per cent.

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- Jour. Am. Med. Assn., September 9, 1911, lvii, 887.
- Jour. Am. Med. Assn., October 27, 1917, lxix, 1394.
- 2 Jour. Biol. Chem., Oct., 1921, xlviii, 373.



## A NOTE UPON THE UTILIZATION OF THE COMPLEMENT-FIXATION REACTION IN THE DIAGNOSIS OF TUBERCULOUS MENINGITIS\*

BY ROBERT A. KILDUFFE, A.M., M.D., PITTSBURGH, PA.

WITH the exception of the meningococcus, the tubercle bacillus is probably the organism most frequently concerned in the production of acute inflammatory reactions in the meninges and, while in all probability, every case is secondary to some primary focus, this is often most difficult to find and, not infrequently, may not be demonstrable.

At autopsy the gross pathology varies within wide limits and may be represented only by lesions barely visible to the naked eye. It is not remarkable, therefore, to find that the results of laboratory examinations of the spinal fluid are also variable and that the diagnosis is often to be made only by exclusion.

As a general rule, the pressure of the cerebrospinal fluid is increased, though this is by no means invariably so, even a normal pressure being not incompatible with the disease. Lack of marked turbidity, a clear, limpid fluid, is a more constant finding but not characteristic of this condition alone, while the formation of a fibrinous, veil-like coagulum in the fluid on standing is highly characteristic though not pathognomonic. The cell count and globulin are both increased, the cytology being characterized by a perceptible and even marked increase in the number of small mononuclear lymphocytes; but, again, these findings, while fairly constant, are only suggestive.

The only absolute method of laboratory diagnosis is the actual demonstration of the tubercle bacillus, a procedure entailing many difficulties and offering varying chances of success—from 50 per cent to 100 per cent according to the method employed and the time consumed in the search. With animal inoculation, even in guinea-pigs previously x-rayed to reduce resistance, ten days is the earliest period in which conclusive results may be hoped for.

Kasahara<sup>1</sup> examines the spinal fluid before and after the intraspinal injection of 0.01 to 0.002 mg. of Old Tuberculin diluted to 1 c.c. with normal saline and, in five cases with three controls, reports, after injection, a marked increase in the number of polymorphonuclear and small mononuclear leukocytes together with, what he considers characteristic, a large number of erythrocytes; but these results have not been corroborated.

The infection, therefore, illustrates in a striking way the necessity for a close correlation between laboratory and clinical findings and is an example of the method of diagnosis by exclusion.

In the case herewith reported, because of the absence of any pathognomonic findings, a tuberculous complement-fixation test was made upon the

\*From the Laboratories of the Pittsburgh Hospital.  
Received for publication, July 28, 1921.

spinal fluid and upon the blood serum and conclusions based upon the following premises:

If the reaction occurred in the spinal fluid, the antigen being biologically specific, the positive reaction would indicate the presence of specific antibodies without, however, indicating their possible source. If, however, the reaction was negative in the blood serum, knowing that the interchange of antibodies between the blood and spinal fluid occurred only in slight degree, then it seemed fair to conclude that the positive reaction in the spinal fluid rose from the presence of tuberculous infection in the meninges. A positive serum reaction would as greatly diminish the significance of a positive spinal fluid reaction as a negative serum reaction enhanced it.

The method was, therefore, tried with successful results in the case reported and the report is made because of the absence of any note in the available literature concerning this method of laboratory diagnosis in tuberculous meningitis.

It is, of course, conceivable that the utility of the method will, in large measure, be determined by the antigen used and the duration of the infection, but it seems worthy of trial in the dubious case, at least.

The antigen used in this case was that supplied by Parke, Davis & Co.; complement being used in two units titrated plain with water-bath incubation at 38° C.

#### CASE REPORT

Mrs. M. F., No. 1319-21, aged forty-four, was admitted to the Pittsburgh Hospital April 18th, 1921, on the service of Drs. Wesley and Williams.

*Chief Complaint.*—Coma.

*Present Illness.*—For several months previous to admission the patient has had severe headaches, the localization of which is not well defined because of the necessity of obtaining most of the history from relatives. These have been accompanied by marked "nervousness." During the attacks she would have crying spells without apparent cause.

The day previous to admission she developed a headache of greater intensity than ever before, became dizzy, and complained that things "became black" before her. She partly recovered from this attack but, at 12.30 A. M. she screamed out, saying that her left side was becoming paralyzed and that she was going to die, and became unconscious, in which condition she was brought to the hospital.

*Past Medical History.*—No illness except measles in childhood.

*Family History.*—Married ten years; one living child. During the "past few years" has been in close attendance upon her husband who recently died from pulmonary tuberculosis, of which there is some family history.

*Physical Examination.*—Face much flushed; pupils pinpoint and slightly oval in shape; equal in size. Some evidence of mastoid tenderness increased on pressure. Patient is mentally fogged, with rambling speech, and generally lies in a comatose condition from which she can be aroused to some extent. In the past 24 hours has vomited frequently, which condition has now subsided. Knee-jerks increased; Babinski and ankle-clonus absent. When aroused complains of severe and agonizing frontal headache.

Heart shows nothing of marked importance. Some tenderness over left kidney on deep palpation; slight tenderness on deep palpation in right iliac region and also in hypogastric region where a small mass is palpable. Tenderness in lumbar region over area corresponding to both kidneys.

April 18, 19, and 20.—Constant headache and rambling delirium. Eye-grounds examined by Dr. Jennings. Negative.

April 23.—Consultation Dr. A. R. Matheny: No evidence of brain-tumor. Diagnosis: Tuberculous meningitis.

April 27.—Examined by Dr. Wright. Diagnosis: Cerebrospinal meningitis, subacute type, most likely tuberculous.

The patient's condition became progressively worse with deepening coma until death on May 10th. Autopsy refused.

*Laboratory Examinations.*—April 18.—Urine negative. Blood: Hb. 80 per cent, RBC: 5,200,000; WBC 19,600. Poly. 80 per cent; L.M. 6 per cent; S.M. 13 per cent; Eo. 1 per cent.

April 19.—Spinal fluid: Bloody; pressure normal. Presence of blood prevented cell count, globulin estimation and gold-sol test. B. tuberculosis not demonstrated.

April 21.—WBC 12,000. Blood Wassermann; negative (Cholesterinized extract human heart; acetone-insoluble lipoids human heart; alcoholic extract luetie liver).

April 23.—Spinal fluid: Bloody; pressure increased. Wassermann negative in 1 c.e. (triple antigen battery).

April 26.—Urine: trace of albumin; otherwise negative.

May 3.—Spinal fluid: Slightly cloudy fluid under increased pressure. Cell count, 300 per c.mm. Globulin increased. Lange colloidal gold reaction, 1100000000. Wassermann negative. B. tuberculosis not found. Tuberculosis complement-fixation test positive in 1 c.e.

May 4.—Blood serum: tuberculosis complement-fixation test negative in 0.2 c.e.

At no time was the tubercle bacillus demonstrated in the spinal fluid.

The report of this case is made possible through the courtesy of Drs. Wesley and Williams and is illustrative of a possible method of laboratory diagnosis in a condition often presenting a puzzling problem concerning which no mention is found in the current literature of the disease.

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## CHAIR FOR PERFORMING SPINAL PUNCTURES\*

BY THOMAS B. CHRISTIAN, M.D., MORRIS PLAINS, N. J.

IN performing spinal punctures in insane patients it is quite different from that of performing spinal punctures among the sane. Most of them do not understand the reason for such procedure and will not cooperate. On account of the resistance it is rather difficult to gain entrance to the spinal canal and when you force an entrance a certain amount of damage is done and the fluid obtained is many times bloody. In different state institutions various methods are used, as putting the patients crosswise on tables, at foot of beds, and

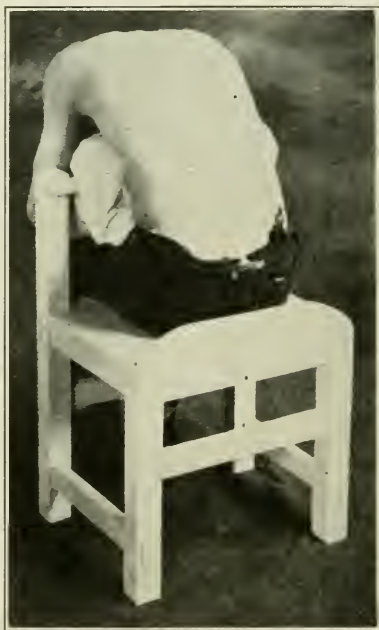


Fig. 1.

sideways in chairs, but this requires too many attendants to hold the resistive patients. We use a chair which is a very simple arrangement and it has the advantages in that only one attendant is required—can be used with the most violent case—and by holding the patient in the right position it diminishes the pain and clear fluids are obtained for examination. As shown in the illustration it is a plain, heavy-made chair with a small elevation in the back to keep the patient from sliding backwards and yet not high enough to interfere with working. The front consists of two heavy posts with a strong

\*From the New Jersey State Hospital at Morris Plains.  
Received for publication, August 8, 1921.



sliding rod. When the patient is seated push the rod down and over it place a pillow and the patient will find it very comfortable to bend over the rod. If the patient is violent a padded strap under his knees and over his neck will keep him in the correct position and it will be impossible for him to interfere with the spinal puncture. With this chair the following advantages have been found, the patient suffers less, painless after-effects diminished, less time consumed, less help required, used with violent cases and clear fluids obtained.

## DEVICES TO ELIMINATE CERTAIN OBJECTIONABLE FACTORS IN THE ANALYSIS OF GASTRIC CONTENT\*

BY J. L. BUTSCH, M.D., AND CLAIRE M. O'BRIEN, A.C., BUFFALO, N. Y.

THE ordinary asbestos piston syringe has some objections, the most important of which are: It is not air tight and does not produce a good vacuum. The plunger must be moistened and in this way dilutes the gastric contents. The piston soaks up about 3 grams of water on an average, and since the specimen collected is usually 5 to 10 c.c. this makes an appreciable

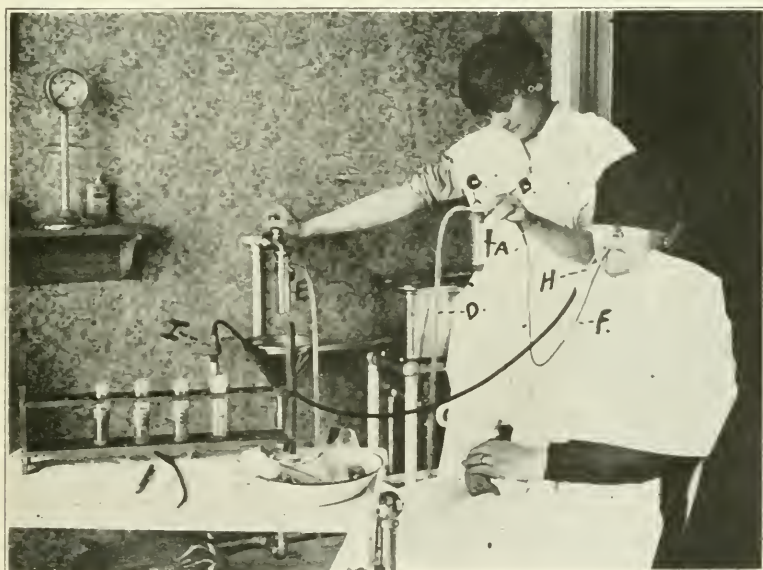


Fig. 1.

difference in the acidity per cent. Where a great many estimations are made the expense of the syringe is a factor. The aspirator method can be done more quickly and there is no necessity for the repeated sterilization of the syringes.

*Procedure.*—An ordinary salt mouthed bottle (A) is fitted with a two-hole

\*Received for publication, August 11, 1921.

rubber cork (*B*) from which pass two glass tubes (*C*) bent at right angles. To one is attached a rubber tube (*D*) which connects it with an ordinary aspirator (*E*), and the other is connected with the sterile Rehfus tube (*F*) which has been introduced by the usual technic. After the tube has been introduced and the connection made, the water tap is turned on and the stomach contents readily flow into the collecting bottle. To collect the second specimen, all that is necessary is to place a fresh bottle onto the cork.

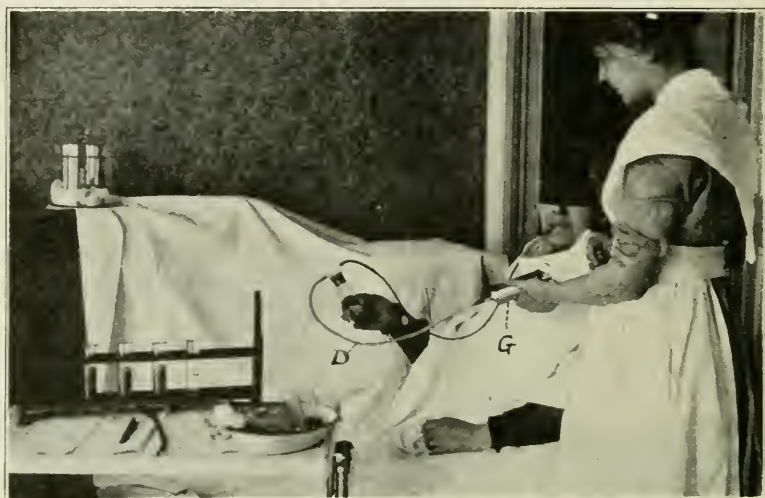


Fig. 2.

The same result may be obtained by connecting a large metal piston syringe (*G*) to the aspirating tube (*D*). To another aspirator (*I*) is attached a curved hook (*H*), on the end of which is a bulb with rather large perforations. This device keeps the excess saliva aspirated away, and there is no occasion for the constant swallowing of the saliva—which also dilutes the gastric contents and neutralizes part of the acidity. By using both devices greater accuracy in check reading is obtained.

# *The Journal of Laboratory and Clinical Medicine*

Vol. VII.

APRIL, 1922

No. 7

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Washington, D. C.

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## EDITORIALS

### *Treatment of Denervated Muscle*

DURING the past few years investigations have been carried on in England and Canada which seriously question the value of electricity and massage in the treatment of denervated muscle. The beneficial effects of such treatment in normal muscle had led to the assumption of a like benefit in denervated muscle. But a denervated muscle, until the connecting of regenerated nerve fibers with the muscle fibers, is probably overactive, judging from the fibrillation which takes place. The discovery of fibrillation should have exploded the old disuse theory as the explanation of denervated muscle atrophy. But it was some time before the old attitude was changed. A normal muscle and a denervated muscle are distinctly different. While the former can be made to atrophy by disuse the latter atrophies apparently from excessive activity. In any consideration of treatment this distinction cannot be emphasized too strongly.

The only experimental work which had been done to test the value of treatment in denervated muscle up to 1915 was that of Reid and that of

Brown-Séquard, both in 1841. These experiments were so few and so incomplete that they established nothing.

Beginning in 1915 Langley and his collaborators in England made a careful study of the effect of massage and of electricity on the atrophy of denervated muscle. After bilateral denervation (popliteal nerves cut) lasting three weeks with treatment on one side, the differences in weights of corresponding muscles were nearly the same as in normal animals. They compared seven or eight muscle groups. The effects of condenser currents, passive movements and massage were tried in different animals. Their results showed no definite effect of treatment. In the conclusion the following statement is made:

"The general impression which we get from our experiments is that none of the methods of treatment of denervated muscle now in use—passage of galvanic current, production of contraction, passive movements and massage—can have more than a slight effect in delaying muscle atrophy."

The effect of treatment in denervated muscle was also studied by Hartman, Blatz and Kilborn. A large number of animals were used so that chance variation might be more easily ruled out. In all animals the contractile power of the muscles was used as a test in order to avoid the connective tissue factor which one does not rule out by a comparison of weights.

In the first series 37 rabbits were used, the soleus, gastrocnemius and plantaris muscles being denervated on both sides. The muscles of the right side were massaged from two to ten minutes a day over periods varying from seven to 190 days. Massage in this and all other series was performed by trained military masseuses. At the conclusion of the experiment the animal was anesthetized and the work capacity of the two sets of muscles compared. The massaged muscles were stronger than the controls in 62 per cent of the animals, but not knowing the relative powers of the muscles to begin with, the results were inconclusive.

A second series was studied in which the contractile powers of the muscles of the treated and untreated sides were tested at frequent intervals before and after denervation. Massage was employed in 58 rabbits and galvanic stimulation in 24 rabbits. These animals were observed for periods ranging from one to nine months. In those in which denervation had been produced by crushing the nerve, there was a return of function long before the termination of the observations. Neither massage nor galvanic stimulation hindered the loss of contractile power nor hastened its recovery when it began to return.

Finally a series of 125 animals was studied in which the ends of the severed tibial nerves were separated in order to check regeneration for periods of time ranging from one to seven months. At the end of this period the two ends were freshened and brought together by suture. The power of the gastrocnemii groups of the two sides was tested at frequent intervals. The denervated muscle group on one side was treated daily by either a slowly surging galvanic current or by massage. Here again neither massage nor electrical treatment appeared to benefit the denervated muscle.

From a study of more than 240 animals and many of these for over a



period of six months Hartman and Blatz conclude that massage and galvanic treatment in denervated muscle are futile.

A certain amount of passive movement is certainly necessary to prevent the development of adhesions but beyond that perhaps the best treatment is rest. In later stages of denervation when the circulation is diminished, hot baths may possibly be of some benefit by increasing the blood supply.

Until we know how to check fibrillation we shall probably not be able to prevent the atrophy of denervation. Neither massage nor electric currents do this except very briefly, therefore it is not surprising that they cannot be shown to be of value.

Still these forms of treatment might be useful after regeneration of the nerve fibers but so far as we know no one has ever been able to show that they delay the muscular atrophy due to denervation.

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—F. A. H.

### *Carotinemia*

**B**Y carotinemia is meant a condition of pseudojaundice which is characterized by the presence of carotin in the blood and certain tissues. It differs from jaundice in that bile is absent from the secretions and from the blood, and in that the characteristic discoloration does not affect the scleræ. The palms of the hands, soles of the feet, and nasolabial folds, cheeks and forehead are according to some reports, more apt to be pigmented. The color is most brilliant in well nourished persons, presumably because the pigment is soluble in fat. When the articles of diet in which the pigment occurs are eliminated from the diet, the color of the patient returns to normal. It seems certain that the xanthosis-diabetica is in fact carotinemia.

Carotin is a yellow pigment belonging with the xanthophylls in the group of carotinoids. To these substances is due the yellow color of cream, of egg yolk, and of corpora lutea. Under different circumstances the pigments are called lipochromes or lutein. They appear in many vegetables, especially in spinach, lettuce, oranges and carrots. Palmer has made a comprehensive study of carotinoids, and with his associates has demonstrated that the natural pigment of egg yolk, body fat and blood serum is physiologically identical with carotin; that the coloring matter of milk fat, body fat, skin secretions, corpus luteum and blood serum of the cow is also due to the same group of substances. They were able to produce milk fat and egg yolk almost lacking in yellow by

feeding diets poor in the respectively characteristic pigments. In the cow the carotin was in excess; in hens the xanthophylls predominated.

In 1919 Hess and Meyers described cases in which there was a peculiar yellow discoloration of the skin and in which carotin was present in the blood. These cases were children who had been fed on a diet containing carrots.

During the war when food shortage necessitated a more largely vegetarian diet, articles appeared in the German journals describing children with yellowish pigmentation. These children regained a normal color when carrots were removed from the diet. Diabetics fed upon food with high lipochrome content have been observed to develop xanthosis. In the vegetable foods used, and in eggs carotin has been found in excess of xanthophylls.

Most recently Head and Johnson have reported a case of carotinemia in an adult who gave carrots an important place in his diet. In the blood serum of this patient carotin was found to amount to approximately 0.00057 gm. per 100 c. c. of blood. Removal of carrots from the diet was followed by an almost complete return to normal color at the end of two months.

Carotinemia is probably commoner than is generally suspected. It is perhaps true that many cases called jaundice, especially ones which appear in persons who prefer a diet composed largely of vegetables, are in reality due to pigments of the carotinoid group. Cases of yellow pigmentation which do not affect the sclerae, in which the color is most intense in the palms of the hands, and in which bile is absent from the urine, should be looked upon with suspicion and studied with reference to their diet.

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—P. G. W.

### *What are Russia's Medical Needs?*

WE have received from the "American Medical Aid for Russia" a request to join in an appeal to the physicians of this country for medical aid in Russia. Naturally, one is sympathetic with requests of this kind and is inclined without question to accept such an invitation. In this case, however, there are certain things about which we think inquiry should be made.

We understand that the U. S. War Department has turned over to the American Relief Association \$4,000,000 worth of medical supplies for distribution in Russia. We are told by an official of the American Relief Association that forty cars of these supplies went into Russia in October, 1921, and from the same informant, we learn that recently \$150,000 worth of laboratory equipment went into Russia. We have in our hands a report on the health situation in Russia made by a committee sent by the League of Nations to Russia, with a statement of affairs up to January, 1922. In this report we are informed that the epidemic of cholera which became so threatening along the Volga has been

arrested, or at least has come to a standstill, in its westward progress. The report says: "The difficult transport situation in Russia assists in the localization of epidemics. Had railway traffic been normal, cholera would have spread westwards. It is surprising, however, that in spite of a fair traffic on the Volga the epidemic does not seem to have traveled very far by this route."

The report says that, according to Professor Taraskevitch, "not a single case of plague has been discovered within the frontiers of European Russia. There have been many suspected cases and all that came to the knowledge of the central or local health authorities were investigated by competent bacteriologists. Just outside European Russia, at Batum in the Caucasus, there was a definite focus of bubonic plague in 1920 with a case mortality of thirty-two per cent. A quarantine station was organized at Irkutsk, and flying laboratories were held in readiness at Moscow, Sebastopol, Saratov, Odessa and Tashkent."

The Health Committee of the League of Nations estimates that there have been 20,000,000 cases of typhus fever in Russia during 1920 and 1921, and it appears that this and relapsing fever have been and continue to be the most threatening epidemic dangers in Russia. There have been local epidemics of dysentery and in some provinces the death rate from this disease, especially among children, has been high. The malarial area in Russia has apparently been greatly extended and is said now to include Archangel. The Russian health authorities, we are told, have no great fear of an extensive outbreak of smallpox.

In this appeal by the "American Medical Aid for Russia," it is said that the intention is to ask for the equipment of an American hospital in Moscow. The report of the Health Committee of the League of Nations contains the following statement: "Nothing struck us so much as the exemplary cleanliness of a big municipal hospital in Moscow (Soldatenkov) which seemed an oasis amidst a desert of rubbish and untidiness surrounding it. All the plant of the hospital was in perfect working order and, in fact, working at full pressure, while the purely professional side seemed to be at a very high level. The staff which has succeeded in keeping this institution in such a state of preservation was being paid salaries much below the famine level, the nurses being paid 5,000 roubles per month and given their food only every second day. The situation of these nurses will be accurately appreciated if it is realized that a cake of soap in a Moscow shop costs 8,500 roubles."

So far as laboratory work and laboratory equipment are concerned, the League of Nations report contains the following paragraph: "In spite of such conditions of life, scientific activity in Russia has not ceased. In fact, one may say that the Russians are perhaps overdoing themselves in their zeal for establishing laboratories everywhere. In Moscow alone there appear to be twelve municipal bacteriologic laboratories, and in the Moscow district there are as many as thirty, while the output of these institutions as regards production of vaccine and serum is rather meagre and the staff very numerous. And yet the scientific researches carried out throughout Russia would do justice to any country. Of course, they have not discovered finally the etiology of typhus. But who has? We should like particularly to mention that we visited a truly magnificent exhibition organized by the Municipal Food Analysis Laboratory

in Moscow, an exhibition which showed the various kinds of bread which have been used by the populations in the various districts of Russia since 1917."

The League of Nations report gives the following statement, which may be of interest: "We have naturally inquired into the needs of the Russian Central Administration. We were given to understand that they were in no need of foreign medical personnel—interpreters would have to be attached to foreign sanitary units whose range of activity was thus rather limited. The People's Commissary of Health did not emphasize the necessity for very large quantities of hospital equipment. He informed us that he could rely on having 125,000 epidemic beds within the frontiers of Soviet Russia, and, in fact, it seems that Russia is at present under better conditions as regards hospital equipment than Poland was in the winter of 1919. This is explained by the fact that large supplies of military hospital stores fell into the hands of the Red Armies after the collapse of Koltechak, Denikin, Wrangel, etc. There is, however, a very great dearth of drugs of practically every kind. Quinine is almost unobtainable, and this at a time when malaria has been spreading at a very alarming rate. There is also a great scarcity of essential instruments such as syringes, etc. There is also room for an almost unlimited supply of soap."

With this more or less contradictory evidence before us, we can only ask, What is the truth about the sanitary condition in Russia?

—V. C. V.



# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, MAY, 1922

No. 8

## *ORIGINAL ARTICLES*

### THE BLOOD-FLOW IN MAN AS ESTIMATED BY THE CALORIMETRIC METHOD OF STEWART\*

By N. B. TAYLOR, M.B., TORONTO, CANADA

A REVIEW OF THE METHODS OF BLOOD-FLOW MEASUREMENT

THE value of dependable blood-flow estimations in man with regard to the investigation of many problems of the clinic as well as of the physiologic laboratory has never, we think, been in doubt. The technical difficulties and limitations of the methods of blood-flow measurement which are at all applicable to the human subject have, however, been great obstacles to the general employment of these methods in the clinic. The plethysmographic method of Brodie and Russell<sup>1</sup> as modified by Hewlett and Van Zwaluwenburg<sup>2</sup> for observations upon man, is useful in estimating, for short periods, the volume flow through the hands. This method is based upon the increase in volume which occurs in the observed part when a pressure sufficient to compress the vein but inadequate to reduce materially the calibre of the corresponding artery, is applied to the limb. The degree of swelling of the limb, which is dependent solely upon the quantity of the inflowing blood, is indicated by a volume recorder whose excursions are calibrated to represent cubic centimeters of blood. By noting the time during which the increase in volume occurs, the blood flow in cubic centimeters per minute can be computed. When used to measure the blood-flow in isolated organs, this method, according to Brodie is very accurate, and the results practically coincide with direct estimations made with the stromuhr. When used to measure the flow in the arm of man, however, the arterial and venous pressures tend soon to become equalized, and in this way an artificial slowing of the blood stream is produced. For this reason it is only for comparatively short periods that

\*From the Physiological Laboratory of the University of Toronto.  
Received for publication, Dec. 2, 1921.

reliable estimations can be made, and the method is inapplicable to prolonged and uninterrupted observations upon blood-flow.

Another method of blood-flow estimation, the gasometric, is commonly employed in physiologic investigations. This is based upon the principle first laid down by Fick which states that if the total consumption of oxygen in a given time be known and the degree to which the oxygen saturation of the arterial blood exceeds that of the venous blood (i.e., the volume per cent of oxygen taken up by the blood in its passage through the lungs) be determined, the amount of blood required to transport this quantity of oxygen may be calculated therefrom. Grehant and Quinquand<sup>3</sup> and Zuntz<sup>4</sup> used this method extensively in animals. Plesch<sup>5</sup> later adapted the method to estimations upon the blood-flow in man and recently its technic has been improved in several respects by Krogh and Lindhard.<sup>6</sup> Though this method possesses a great measure of accuracy, the complexity of the procedures necessary to carry it out, and the exacting nature of its technic, render it impracticable for routine employment in the clinic. Furthermore, such estimations give the amount of blood circulating through the lungs or through the entire body in a given time and though of great value in the determination of the minute volume of cardiac output, do not give us any information regarding local changes in blood-flow or enable us to learn the manner in which certain physiologic or pathologic states, or various therapeutic measures affect the flow through the peripheral vessels.

For these reasons we have considered the calorimetric method as devised by G. N. Stewart<sup>7</sup> to be the most suited to our purpose. In this, the flow through the hand (or foot) is computed from the heat which the blood coursing through the vessels of the part gives off to a known body of water contained in a calorimeter and in which the hand or foot is immersed. By this method the blood-flow can be estimated practically uninterruptedly over prolonged periods, and yet the method is sensitive enough to register fairly abrupt changes in flow throughout the course of an experiment. The technic once mastered is not difficult or burdensome and requires no special laboratory training for its practice. This method, however, has in common with that based upon plethysmography, the obvious disadvantage that its application is limited to those parts of the body which are readily accessible, namely the hands and feet.

#### DESCRIPTION OF THE CALORIMETRIC METHOD

The part under investigation is placed in a specially designed calorimeter. This consists of a cylindrical water-tight copper vessel 8" in diameter and 12" deep. Its upper end is closed save for one large oval opening 3" × 4" in diameter through which the hand is admitted, and two smaller ones, one for the admission of a thermometer graduated in  $\frac{1}{10}^{\circ}$  C., and the other for the passage of a metal tube which conveys the compressed air used as the water-mixing agent. The copper cylinder is contained in a larger vessel 16" in diameter and 14" deep, constructed of galvanized sheet metal. The two vessels are separated from one another by granulated cork to ensure a high

degree of heat insulation. A cork slab 1" thick, in which orifices corresponding to those present in the inner copper vessel are cut, closes the upper end of the larger vessel. To reduce heat loss to the lowest degree possible, a jacket made of absorbent cotton 1" thick contained between two layers of gauze, is fitted snugly to the outer vessel so as to cover its top, sides and bottom. That these measures are efficient for all practical purposes in rendering the apparatus heat-tight is shown by the fact that the cooling of the water in the calorimeter amounts, at average room temperature to only  $.1^{\circ}\text{C.}$   $.12^{\circ}\text{C.}$  in ten minutes, when its initial temperature is  $33^{\circ}\text{C.}$

The calorimeter as originally designed by Stewart was provided with a pair of goose feathers to serve as stirrers. The observer, by imparting alternately up and down and to and fro movements to the latter, which were admitted to the interior of the calorimeter through two small openings in its cover, endeavored to maintain a constant agitation of the water therein, with the object of securing thorough mixing. We have found, however, such stirrers to be unsatisfactory in that fluctuations in the readings of the thermometer occurred—unless the movement was very vigorous—which we ascribed to imperfect mixing. Moreover, when readings are taken every minute from two thermometers, as is the case when each hand is placed in a calorimeter, the work of the observer becomes unduly irksome.

For these reasons we have employed compressed air, of suitable temperature and vapor pressure, as the stirring agent. This was conveyed by rubber tubing from the compressed air supply of the laboratory to a flattened coil of lead pipe  $\frac{1}{4}$ " in calibre and pierced by pin-holes at intervals of one to two inches. The coil was placed within the inner vessel of the calorimeter where it rested upon the bottom so as not to interfere with the position or movements of the hand. The air issuing from the pin-holes produced numberless small bubbles which ascended throughout all parts of the liquid. The supply of air was so adjusted that the agitation of the water was very gentle. Though this was so, the mixing was continuous and most efficient. Observations upon heating of the water when air-mixing was in operation and when, instead of the hand being inserted, a *constant* source of heat, for instance an electric lamp, was immersed in the water, showed a steady rise of temperature without fluctuations. The curves plotted from such observations were practically straight lines.

In order that there should be no increase of heat loss through vaporization of the water as a result of this ebullition, the air, before its entrance into the calorimeter was made to bubble through a body of water equal in depth and of the same temperature as that in the calorimeter. This body of water which served as an adjuster of vapor tension was contained in an Erlenmeyer flask of 2 litres capacity. As an assurance that the temperature of the entering air should be neither higher nor lower than the calorimeter temperature and should, therefore, neither add to nor diminish this temperature, the air was passed through a copper worm of many turns immersed in a body of water at calorimeter temperature (i.e.,  $30$  to  $33^{\circ}\text{C.}$ ); and contained in a large galvanized iron vessel of 6 litre capacity. This vessel was insulated by granu-

lated cork and absorbent cotton in a manner similar to that employed for the calorimeter. As a means of economizing space the copper worm was arranged so as to embrace the Erlenmeyer flask and the whole immersed in the water.

Each calorimeter was provided with a thermometer graduated to  $\frac{1}{10}^{\circ}$  C. Its bulb dipped below the level of the water. By means of a powerful convex lens placed a few inches in front of the thermometer and a telescope at a distance of 5 feet or so, the graduations were so magnified that readings to  $\frac{1}{100}^{\circ}$  C. could be judged with a fair degree of accuracy. Felt cuffs were employed to encircle the wrist and seal any space between the forearms and the calorimeter opening.

The calorimeters as originally designed and at present in use in this laboratory, are inconvenient for clinical work, especially for cases confined to bed and seriously ill. This is due to the fact that their construction provides no protection against leakage of water when in any but the upright position, and in a recumbent patient it is most difficult to get the apparatus low enough to enable the hand to be inserted. Simultaneous observations upon the two hands, on account of the width of the bed, is quite out of the question. We are at present engaged in the construction of a calorimeter which will enable it to be placed upon its side without leakage. This has been made possible through the employment of a specially designed rubber sleeve, which, though water-tight offers, we believe, no appreciable obstruction to the blood-flow. A description of the details of this apparatus is reserved for a future communication.

#### MODE OF PROCEDURE IN TAKING A BLOOD-FLOW OBSERVATION

A known quantity of water usually 2500 to 3000 c.c. at a temperature of approximately  $30.2^{\circ}$  C. is placed in the calorimeter. The hand of the subject, who is seated comfortably in a chair of adjustable height on one side of the apparatus, is placed in the calorimeter and submerged in the water to an arbitrary line previously marked upon the wrist (usually at the level of the styloid process of the ulnar) by a blue pencil. The arm should hang from the shoulder in an easy position without discomfort or straining. The hand is kept immersed for a period of ten minutes during which time no readings are taken. The felt cuff is not in position during this time. This initial ten minute period is for the purpose of permitting the temperature of the solid tissues of the hand to assume a steady relationship to that of the surrounding water. Stewart employed a separate bath for this purpose but we have found it simpler to place the hand directly in the calorimeter for the initial immersion. At the termination of this period the blue line on the wrist is more precisely adjusted to the level of the water, and the felt cuff fitted snugly to the wrist in order to close effectually any space between the latter and the margins of the opening, and so render the apparatus as nearly heat-tight as possible. Care should be taken, however, that the cuff is not applied so tightly as to obstruct the venous return. The compressed air is now turned on and readings of the thermometer are commenced and taken at one minute intervals for ten minutes. At the conclusion of this period the hand is removed and



the oval opening in the calorimeter closed by a felt disc. Readings at one minute intervals are continued for ten minutes longer in order to determine the rate of cooling of the calorimeter. The volume of the hand is measured by placing it in a graduated vessel containing water. The amount of displaced water gives the volume of the hand. In cases where an observation is taken upon one hand only, the opposite hand should be kept wrapt up to protect it from draughts. The temperature of the patient is taken in the mouth, either at the commencement or at the termination of the experiment. The pulse rate is usually taken both before and after the observation.

#### CALCULATION OF THE BLOOD-FLOW FROM THE DATA OBTAINED FROM THE FOREGOING PROCEDURES

The grams of blood flowing through the part during the course of the experiment are calculated from the following formula:

$$Q = \frac{H}{T - T'} \times \frac{1}{S}$$

where Q represents the quantity of blood in grams, H the heat given out to the water of the calorimeter, T and T' the temperatures in degrees centigrade of the arterial and venous bloods respectively, and 1/S the specific heat of blood.

The factor H is the product of the weight of the water of the calorimeter in grams by the degrees centigrade through which the temperature of the water is raised. Since the inner vessel of the calorimeter and the tissues of the hand itself are raised in temperature, the mass of these must be translated into terms of water (water equivalent). The inner vessel upon determination has been found to have a water equivalent of 100 grams. The water equivalent of the hand is taken as its volume multiplied by 0.8. This latter figure is a constant arrived at by Stewart from separate experiments; it is the product of the specific heat of the hand by its specific gravity. (In the case of the foot a slightly lower figure is used.) H, therefore, is made up of several components. Employing the data of an actual experiment this factor may be expressed as follows:

H	Wt. of	water equiv.	water equiv.	degrees rise	cooling
in gram	= water	of cal.	of hand	in temp.	of cal.
calories	(2500	+ 100	+ (400 x 0.8))	x (.5	+ .12)

Since the blood flowing through the hand is the only source of this heat (for the heat generated by the solid tissues is inappreciable), it follows that if we can determine the number of degrees centigrade that the temperature of the blood has fallen in its passage through the tissues, i.e. from the arteries to the veins, we may arrive at the number of grams of blood coursing through the part by dividing this factor into H. This temperature drop is represented by  $T - T'$ . The mouth temperature is taken as the temperature of the arterial blood. (T). The venous temperature (T') has been shown by Stewart to be that of the average temperature of the water in the calorimeter throughout the course of the investigation. This implies that the hand is a perfect radiator, the blood giving up its heat readily to the surrounding medium.

The specific heat of blood as represented by 1/S is taken as .9.

Since the blood flow is more conveniently expressed in grams per 100 c.c. of tissue per minute, the result, as obtained from the foregoing data is divided by the duration

of the observation in minutes and by the volume of the hand over 100 ( $\frac{V}{100}$ ).

## RESULTS

*Normal Flow.*—A large number of observations have been made upon the blood-flow of apparently normal individuals. The subjects were of both sexes and young, their ages varying from twenty to thirty-five years. In the majority of the cases, observations were made simultaneously upon the two hands, readings being taken every minute for periods of ten minutes. The temperature of the room used for the investigations was kept constant from day to day during the winter months, the daily fluctuations as a rule not exceeding 1 or two degrees in magnitude. In order to eliminate the influence of the outside temperature, the subject spent a period of one hour or more in the observation room, or at least in a room of the same temperature, before the observation was commenced. In summer no measures were taken to regulate the room temperature, it consequently varied directly with the temperature of the day. The features which stand out prominently as an outcome of this series are :

(1) The marked differences in the volume of the blood-flow in different individuals subjected to the same conditions.

(2) The variability of the flow, in relation to the temperature of the outside air, in the same individual.

(3) The fluctuations in the flow in the same individual during the course of an experiment.

We shall consider these different features of the normal flow in order.

(1) *The differences in the volume of the blood-flow in different individuals subjected to the same conditions.*—The flow has been found to vary from 1 gram per 100 c.c. of hand tissue per minute in some individuals, to 12 grams or more per 100 c.c. per minute in others, when the room temperature, time of day, and the interval following a meal, were approximately the same in all cases. Since the right hand almost invariably showed a higher blood-flow (from 10 per cent-20 per cent), the flow in hands of the same side were always compared.

The series of experiments upon which these conclusions are based was conducted during the winter months, and it was insisted that the subjects should be in the building for from one to two hours before the test was commenced, and that the hand should be in the calorimeter for ten minutes before the first reading was taken. Several observations (5 or more) were taken upon each individual. The duration of the observations varied from ten to thirty minutes, the duration of the majority approaching the latter figure. Readings were taken every minute and the blood-flow computed for five-minute periods. Even though the hands had been in the calorimeters for ten minutes before the actual estimations were made, the first few readings were sometimes erratic, the temperature not rising by equal increments in successive one-minute periods. Such readings were discarded as undependable, only those being taken after a steady rise had been reached. By calculating the results in five-minute periods minor errors, due to inequality in temperature rise, or in thermometer readings, were minimized. Calculation of the

TABLE 1

COMPARISON OF BLOOD-FLOW IN DIFFERENT NORMAL INDIVIDUALS  
(in gms. per 100 c.c. of tissue per min.)

SUBJECT	DATE	PERIOD OF OB- SERV.	MAX. FLOW	MIN. FLOW	AVERAGE FLOW DURING ENTIRE PERIOD	ROOM TEMP.	TEMP. OF DAY	RELATIVE HUMIDITY	BAROMETRIC PRESSURE
			Mins.	Grams	Grams	°C.	°C.	%	In mm. of Hg.
J. C.	20/2/20	30	2.1	0.5	1.3	22.2	-1.8	73	757
	8/3/20	20	2.5	0.6	1.7	23.3	-4.1	58	754
	20/3/20	20	2.0	0.1	1.1	22	-1.7	65	753
	30/3/20	10	2.2	1.3	1.7	22	4.9	62	747
- C.	13/8/19	30	-	-	1.8	20	20	-	—
	19/8/19	30	-	-	2.7	21.5	21.5	-	—
	18/9/19	30	-	-	0.7	16.5	16.5	-	—
R. H.	24/2/20	30	13.1	7.7	10.7	22	-4.7	74	744
	9/3/20	30	25.0	18.3	21.2	26	3.2	69	754
	9/3/20	30	27.4	20.8	23.6	26	2.6	72	753
	16/3/20	30	11.5	2.4	7.0	21.8	7.3	74	741
	24/3/20	30	10.3	2.8	5.0	20	15.8	33	753
	25/5/20	10	13.0	11.4	12.2	23.8	25.6	59	748
	2/6/20	10	7.6	2.2	4.9	19	19.5	60	755
S. P.	9/4/20	20	7.3	5.4	6.4	25	1.4	42	749
	3/6/20	10	12.3	7.3	9.8	23	22.4	27	754
	7/6/20	10	3.3	3.2	3.3	19.5	22.4	35	753
	15/6/20	10	9.7	7.6	8.6	23.5	20.8	92	744
	16/6/20	10	7.4	5.6	6.5	20	17.4	79	743
	29/9/20	10	6.3	3.8	5.0	19.2	10.3	86	749
	13/10/20	10	6.1	5.7	5.9	20.5	17.5	77	753
	15/10/20	10	5.6	4.4	5.0	21	21.4	61	754
D. H.	26/2/20	30	9.8	5.2	7.8	22	-12.9	63	748
	19/10/20	30	2.2	0.6	2.0	18	14.1	96	755
		30	8.2	5.1	6.3	22.5			
	22/10/20	30	8.0	6.4	7.2	22.5	20.8	77	755
	22/10/20	10	8.7	7.1	7.8	22.5	20.8	77	755
	25/10/20	10	4.2	1.9	3.0	20	14.9	62	752
	1/11/20	10	6.1	5.0	5.5	22.2	4.4	82	760
	2/11/20	30	6.2	1.6	3.7	20	10.5	99	760
K. H.	26/3/20	10	7.8	6.5	7.2	21	17.2	73	737
	31/3/20	10	5.4	2.2	4.0	19.2	7.1	72	749
	7/4/20	10	17.8	11.3	15.2	23	1.1	65	743
	13/4/20	10	14.4	11.1	12.7	21	3.7	58	741
	21/4/20	10	15.0	8.4	12.4	20.3	6.8	99	740
	13/4/20	10	14.0	9.3	12.2	21	3.7	58	741

average flow throughout the entire list was based upon these five-minute estimations. Table I will illustrate the differences in six individuals. It is seen that J. C. exhibits a very meagre flow, and though in good health and normal in every way, consistently showed a flow of from 1 to 2 grams per minute. Only on two occasions out of 8 did the average flow exceed, or even reach, 2 grams per minute. In both these instances the subject previously had unwittingly performed light muscular work. All the observations were made at low out-of-doors temperatures. R. H. shows the greatest flow. The extremely large flow in two observations on this subject is no doubt due in part to the unusually high room temperature. Even for this temperature,

however, the flow is considerably higher than the average. S. P. for instance shows a flow of only 6.4 gms. when the room temperature was 25° C.

(2) *The variability of the flow in relation to the temperature of the outside air.*—In the summer and early fall when the temperature of the observation room was the same as the outside air, the rise and fall of the blood-flow in relation to the outside temperature was most definite and pronounced. In winter, on the other hand, when the observation room was artificially heated, the temperature of the atmosphere of the room at the time of the experiment was found to have little influence upon the blood-flow unless the subject was resident in the heated room for a considerable time. The temperature of the outside air was the dominating influence and remained so for at least two hours after the subject's entry into the building. The following curves

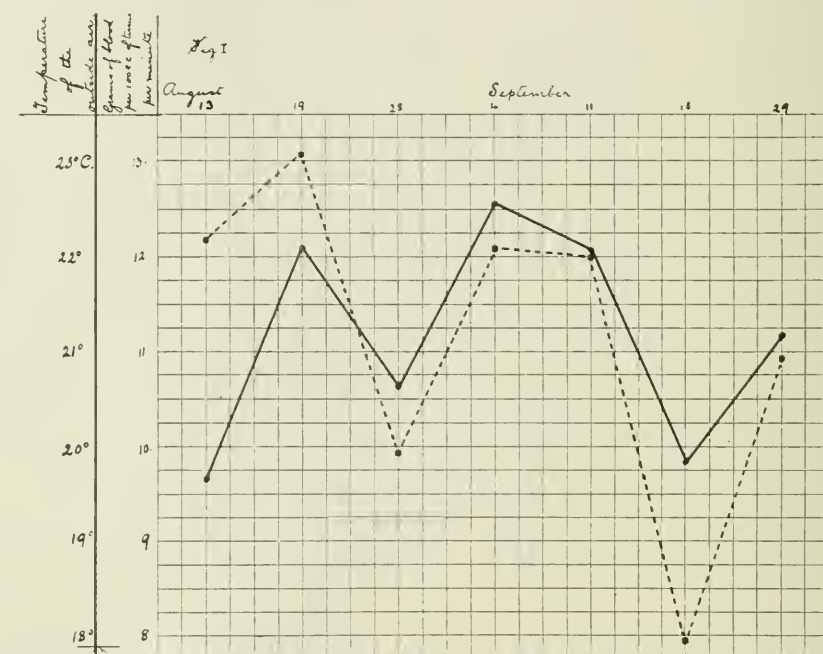


Fig. 1.—Showing the influence of temperature upon the blood-flow. Continuous line=blood-flow; broken line=temperature of outside air.

(Fig. 1) illustrate the (direct qualitative) relationship which peripheral blood-flow bears to atmospheric temperature. These figures are also given below in tabular form (cf. Table II).

The continuous line represents the blood-flow in grams per minute per 100 c.c. of hand substance. The broken line represents the temperature of the day in degrees centigrade. The days of the month for both curves are indicated along the abscissæ.

In some instances as in the case of C-, a quantitative relationship between blood-flow and atmospheric temperature is seen to exist. In the following Chart (Fig. 2) this is shown, the daily temperatures are plotted along the abscissæ and the grams of blood along the ordinates. The graph shows that



a rise of  $1^{\circ}$  C. causes a much greater change in blood-flow as the temperature increases. There is reason to believe that the deviations of the curve from a smooth line are dependent upon variations in the cooling influence of the atmosphere apart from its temperature, for we have found a relationship to exist between the humidity and the blood-flow. Owing, however, to the limited number of our observations, we are unable at present to make any definite assertion in this regard. The fluctuations in the curve were not found to bear any relationship to the barometric pressure.

(3) *The fluctuations in flow in the same individual during the course of the experiment.*—One of the outstanding features of the blood-flow estimations

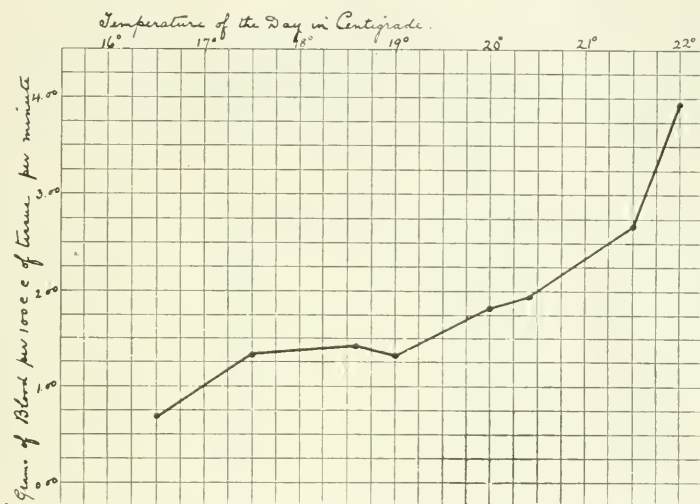


Fig. 2.—Showing the influence of the temperature of the outside air upon the blood-flow.

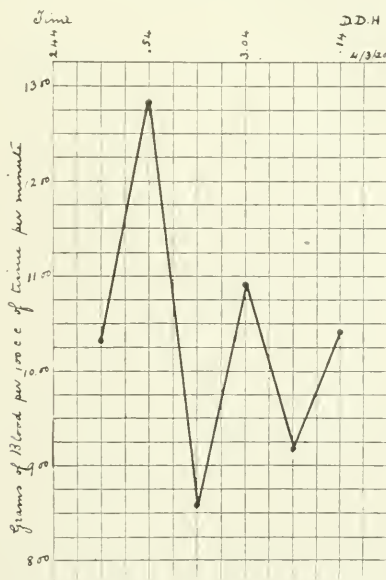


Fig. 3.—Showing fluctuations in the blood-flow during the course of an experiment.

in all the individuals examined was the marked variability in the volume of the flow, that occurred within a comparatively short period of time. The augmentation or diminution in the flow which occurs above or below the mean, is of considerable magnitude, amounting in some cases to as much as 20 or 50 per cent of the latter. The following curve and table illustrate this well (Fig. 3 and Table III.) In this curve the flow had been estimated for five-minute periods. When ten minute estimations are made the fluctuations disappear to a large extent, indicating that they occur at a certain rate and are of a definite duration.

TABLE II

SHOWING THE INFLUENCE OF THE TEMPERATURE OF THE OUTSIDE AIR UPON THE BLOOD-FLOW

SUBJECT	PART OBSERVED	DATE OF OBSERVATION	OUTSIDE TEMPERATURE, IN DEGREES CENTIGRADE, AT TIME OF OBSERVATION	GRAMS OF BLOOD PER 100 C.C. OF TISSUE PER MINUTE
C-	L. hand	13/8/19	20	1.8
"	"	19/8/19	21.5	2.7
"	"	28/8/19	17.5	1.3
"	"	4/9/19	20.4	1.9
"	"	11/9/19	19	1.3
"	"	18/9/19	16.51	0.7
"	"	25/9/19	18.6	1.4
"	"	30/9/19	22	3.9
W-	Rt. hand	13/8/19	22.2	9.7
"	"	19/8/19	23.1	12.1
"	"	28/8/19	19.9	10.65
"	"	4/9/19	22.1	12.55
"	"	11/9/19	22.0	12.10
"	"	18/9/19	18.2	9.8
"	"	29/9/19	20.9	11.2

The fluctuations could not be accounted for by changes in the temperature of the room, psychologic influences (special precautions having been taken against the latter), variations in the pulse-rate or the presence of draughts. In all experiments the room temperature remained practically constant throughout and the subject was guarded against draughts which might produce a local depression of temperature. The pulse rate was taken before and after the observation and in most cases showed little or no alteration. As usual the calculations were made from readings taken after the thermometer assumed a steady rise in temperature. If the earlier readings showed irregular rises they were discarded. The hand in all cases, it is to be recalled, remains in the calorimeter for ten minutes before the readings are commenced. The variations in flow were not of the same degree in all subjects, some showing much greater fluctuations than others. This may indicate a greater or less stability of the vasomotor system in different individuals. One may only speculate with regard to the cause of these fluctuations. They are possibly due to automatic discharges from the vasomotor centres, but on the other hand they may be of a reflex nature, and dependent upon some changing condition which we have so far been unable to discern. Practically all the subjects examined showed a decided decline in the volume of the blood-flow toward the latter part of the observation, unless this was of short duration.

This fact, already remarked upon by Stewart, will be made evident also from consultation with Table III and Fig. 3. The improbability of the fluctuations being due to faults in the method of estimation and of being independent of alterations in the blood-flow itself, was shown by a series of observations taken over prolonged periods, upon the cooling of the water in the calorimeter when the hand was not immersed. The conditions under which this series of tests was carried out were identical with those under which the blood-flow measurements were conducted; the method was employed in precisely the same way. These tests of which 15 were made, were of one hour's duration, readings being taken every minute over this period. The graph plotted from the changes in temperature recorded each minute over the entire period, is a straight line (see Fig. 4, interrupted line).

The converse experiment, namely, of placing a *constant* source of heat, such as a lighted electric lamp, beneath the water of the calorimeter, and

TABLE III

SHOWING FLUCTUATIONS IN THE BLOOD-FLOW DURING THE COURSE OF AN EXPERIMENT

SUBJECT	DATE	PART OB- SERVED	TIME	GMS. BLOOD PER 100 C.C. OF TISSUE PER MIN.	ROOM TEMP. ° C.	SUBJECT	DATE	OB- SERVED	TIME	GMS. BLOOD PER 100 C.C. OF TISSUES PER MIN.	ROOM TEMP. ° C.
-W.	13/2/20	L. Hand	P. M. 4.20 .25 .30 .35 .40	13.0 8.3 13.7 10.5 9.2		E. J.	5/3/20	L. Hand	P. M. 3.50 .55 4.00 .05 .10 .15	8.4 6.0 6.8 7.0 3.7 5.6	23.7°
J. C.	20/2/20	"	3.27 .30 .33 .36 .39 .42 .45 .48 .51 .54	1.1 0.5 2.1 1.6 2.1 1.6 1.6 1.1 1.1 0.8	22.2°	D. D. H.	4/3/20	"	2.49 .54 .59 3.04 .09 .14	10.3 12.8 8.6 10.9 9.2 10.4	24.5°
K. H.	25/2/20	"	3.50 .55 4.00 .05 .10 .15	9.1 9.4 8.5 9.5 6.5 3.7	25°	- S.	8/3/20	"	3.50 .55 4.00 .05 .10 .15	16.1 12.0 15.6 14.8 9.4 10.1	24°
N. B. T.	26/2/20	"	12.06 .11 .16 .21 .26 .31	6.0 4.7 5.0 4.7 3.7 5.1	21°	R. H.	9/3/20	"	2.45 .50 .55 3.00 .05 .10	25.6 22.5 27.4 23.5 21.9 20.8	26°
E. J.	27/2/20	"	3.13 .18 .23 .28 .33 .38	6.9 9.1 6.5 5.1 7.8 9.5	23°	M. S.	10/4/20	"	12.14 .19 .24 .29 .34 .39	3.9 3.2 5.1 3.1 3.7 3.4	24°

recording each minute the temperature rise, was also performed. A graph plotted from these observations is also practically straight (Fig. 4, continuous line), but is of course an ascending one.

That the fluctuations in the blood-flow curves are well within the limits of experimental error is also evident from the calculations given below. As stated elsewhere, the thermometers used were graduated to  $0.1^{\circ}$  C. By magnification, readings to 0.01 could be judged. The probable error resulting from the thermometer readings, when five minute computations of flow were made, has been estimated to be  $0.02^{\circ}$  C. It is seen from the following equation that a difference in temperature of  $0.02^{\circ}$  C. would represent a difference

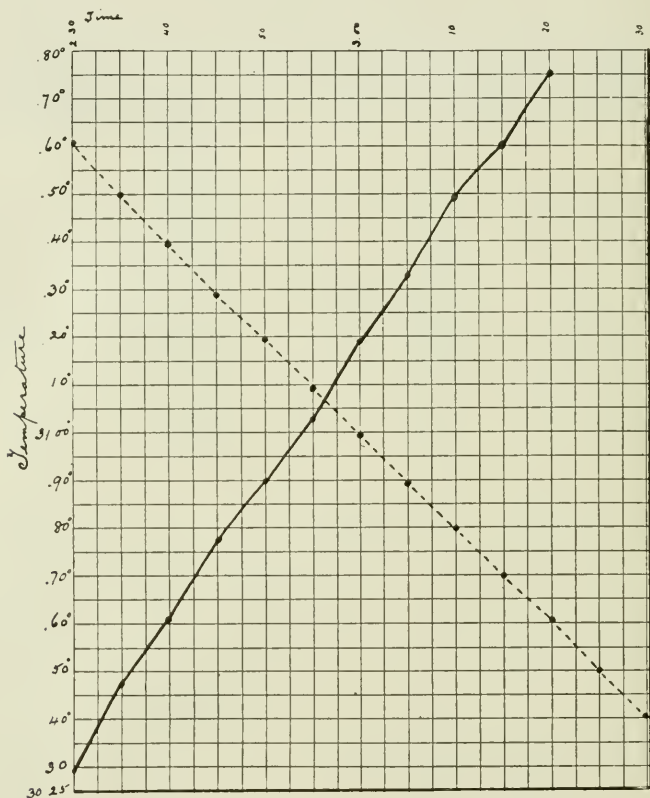


Fig. 4.—Curves of temperature rise and fall. Dotted line joins readings taken during cooling of calorimeter and the continuous line those while it was being warmed by an electric lamp submersed in the water.

in blood-flow of about 0.5 of a gram only, whereas the variations in flow shown in Table III and curve three are of a magnitude of several grams.

$$\frac{2500 + 100 + (400 \times 0.8) \times 0.02}{4 \times 5 \times (37.00 - 30.50)} \times \frac{10}{9} = 0.49 \text{ grams per 100 c.c. per minute.}$$

Mean values from a large number of observations were taken as the data for the hand volume, and for the venous and arterial temperatures, in the foregoing equation. For the production of the large variations shown in some of these curves, an error of from  $.1^{\circ}$  to  $.2^{\circ}$  C. in the thermometer readings would



have to occur. It is quite impossible that an error even approaching this figure could exist.

*Observations upon the reflex changes in blood-flow through the hands following certain local measures, e.g., applications of hot and cold water, and draught.*—For the sake of greater convenience and in order to eliminate certain technical difficulties the great proportion of observations upon the reflex changes in blood-flow in the two hands was made by placing one hand only in the calorimeter and estimating the blood-flow through it while the particular measure, the effect of which was under investigation, was directed to the opposite hand. Such a procedure implies that the blood-flow in the two hands responds similarly when a given stimulus is applied to one, in other words, the homolateral is taken to be identical with the contralateral reflex. We believe

TABLE IV  
VASCULAR REFLEX TO COLD

SUBJECT	DATE	GRAMS OF BLOOD PER 100 C.C. OF TISSUE PER MINUTE						ROOM T. °C.	MOUTH TEMP.	PULSE RATE PER MIN.
		RIGHT HAND			LEFT HAND					
		A	B	C	A	B	C			
J. E.	16/3/21	10.8	—	4.7	8.9	8.5	8.7	23°C.	36.8°C.	73-68
*	30/3/21	4.4	3.0	4.2	5.2	4.1	5.6	17°	36.7°	71-64
*	4/4/21	11.1	8.2	2.5	10.0	6.8	6.3	19.6°	37°	80-70
D. D. H.*	16/3/21	9.7	1.2	5.0	9.5	2.7	2.4	23.8°	36.8°	89-84
*	21/3/21	4.6	2.6	0	2.2	1.3	1.8	20.4°	36.7°	88
*	24/3/21	6.1	2.2	0.2	4.7	1.3	1.9	19.7°	37.2°	80-77
	5/4/21	3.5	2.4	2.8	4.6	4.4	3.7	21°	37.1°	93-80
*	11/4/21	7.3	6.3	0.4	6.1	3.0	2.2	20°	36.6°	93
J. L.*	17/3/21	7.3	4.0	1.1	8.7	7.8	7.4	21.5°	36.8°	95-84
	21/3/21	8.3	2.6	3.6	5.5	5.9	4.5	20.1°	36.7°	92-88
	31/3/21	6.2	3.6	2.6	5.2	5.0	6.6	20.3°	36.5°	78
G. D.	18/3/21	9.3	3.1	0	10.7	7.2	8.2	19°	36.7°	96-92
*	1/4/21	8.6	5.1	3.4	8.3	6.1	6.0	17°	37.2°	90-92
*	5/4/21	13.5	5.9	5.1	12.2	8.1	10.7	21.2°	37.3°	100-96
N. R. H.	28/3/21	7.4	5.9	6.1	4.8	4.7	4.4	17.4°	37.7°	78
*	1/4/21	2.5	2.9	0.6	2.5	2.1	1.9	17.3°	36.6°	82-80
	4/4/21	9.8	8.7	4.8	8.8	5.2	4.6	19.1°	37.1°	88-80

A = normal flow for 10".

B = Right calorimeter emptied and filled with water at about 10° C.

C = Right calorimeter emptied and refilled with water at about 31.5° C.

that such a conclusion is warranted by our experiments. The graphs in Fig. 5 were plotted from an experiment in which both hands were placed in calorimeters and blood-flow estimations made upon each while the temperature of the water in one calorimeter (left) was lowered to 10° C. It is seen that though the two curves are at slightly different levels, they follow roughly the same course. The opposite hand, it is seen, has responded rather more promptly to the excitant; this, however, is not the case as a rule. The experiment which supplied the data for these curves was a protracted one and there appears to be a tendency for the flow to rise toward the end of the observation. This may indicate a reduction of constrictor tone due to fatigue.

Table IV was compiled from a series of experiments carried out with a view to determine the response of both hands when the water in one calorimeter only was lowered. The other calorimeter contained water at the

usual temperature of  $30^{\circ}$ - $31^{\circ}$  C. The results, however, do not show in all cases similar responses in both hands. The experiments marked with an asterisk alone show a similar response in the two hands, but even in these, the response of the contralateral hand was not so marked as those obtained for similar temperature differences in later experiments when the flow in one hand only was measured. The inaccuracies involved in measuring the blood-flow of one hand while it was immersed in water at  $10^{\circ}$ , instead of the usual 30 to  $31^{\circ}$ , we believe are responsible for these differences. It will further be observed that the curtailment of flow persists during the ten minute period (C) following that of the application of cold.

In order to avoid these difficulties in determining the relative responsive-

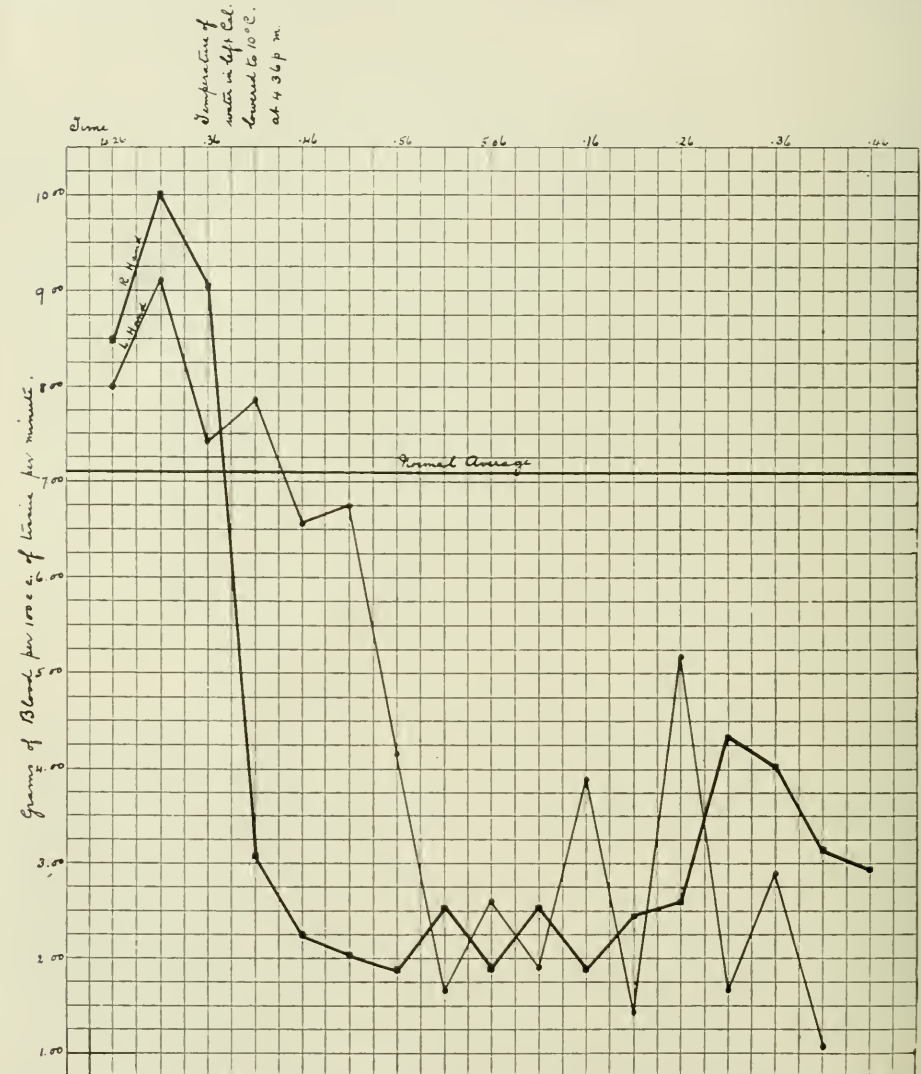


Fig. 5.—Vascular reflexes to cold. Thin line gives blood-flow in left hand placed in a calorimeter, the temperature in which was lowered by adding cold water. The thick line gives the blood-flow in the opposite hand.

ness of the hands, instead of using cold water as the cooling agent, we have employed a draught produced by an electric fan. Blood-flow measurements were made upon both hands placed in calorimeters with water about  $31^{\circ}$  and a fan allowed to play upon one arm only, the opposite arm being shielded. Table V shows that the contralateral hand responds to the air current as promptly, and to very nearly the same degree, as the hand on that side to which the draught was directed. Experiments were also performed to decide whether

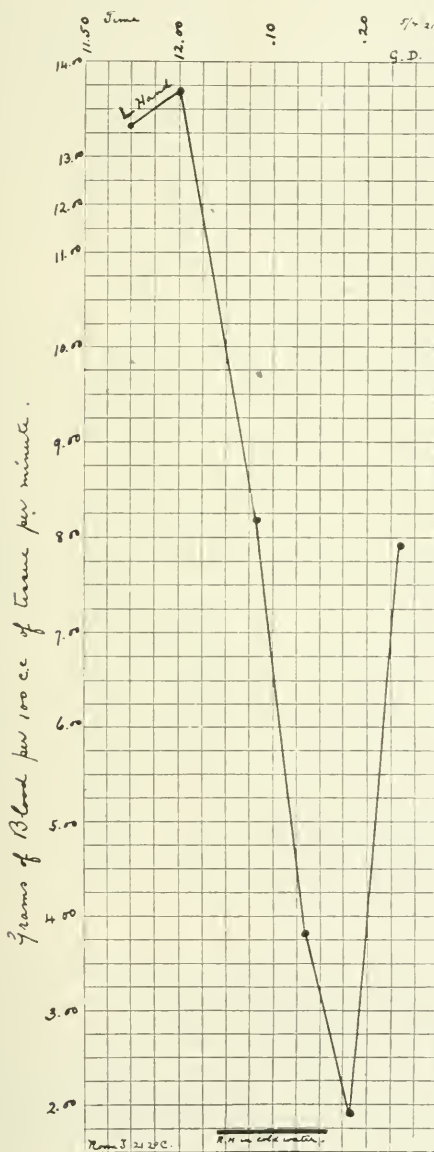


Fig. 6.

Fig. 6.—Vascular reflex to cold. Flow through left hand before, during and after immersion of the right hand in cold water.

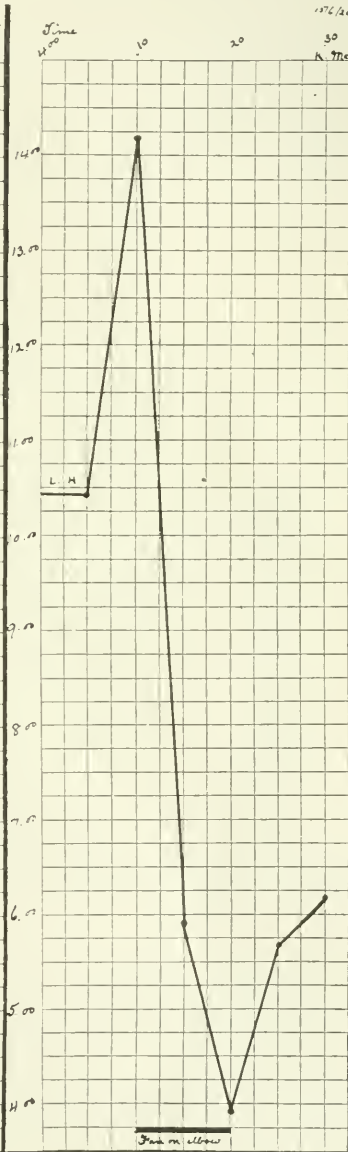


Fig. 7.

Fig. 7.—Vascular reflex to draught. Flow through left hand before, during and after allowing a fan to blow on the exposed elbow of the same side.

both hands responded similarly when heat was applied to one. In all cases when one hand was placed in hot water an increase in blood-flow through both hands occurred, the increase bearing practically the same ratio to the normal in the two hands. Owing to the close agreement of the blood-flow in the two hands the estimations in the following experiments were made upon one hand alone.

(1) *Effect of the local application of cold water to one hand upon the blood-flow in the opposite hand.*—The following curve and Table (Fig. 6 and Table VI) illustrate the effect of cold upon the blood-flow in the hand. The right hand was placed up to the wrist in a basin of water at from 5°-8° C. and kept immersed for a period of ten minutes. Readings upon the flow through the left hand, placed in the calorimeter were taken each minute. A preliminary observation of ten minutes upon the blood-flow in the left hand was made prior to the immersion of the right in the cold water, in order to obtain

TABLE V  
VASCULAR REFLEX TO DRAUGHT

SUBJECT	DATE	GRAMS OF BLOOD PER 100 C.C. OF TISSUE PER MINUTE						ROOM TEMP. °C.	MOUTH TEMP. °C.	PULSE RATE PER MIN.
		RIGHT HAND			LEFT HAND					
		A	B	C	A	B	C			
		10''	10''	10''	10''	10''	10''			
J. E.	30/5/21	16.1	4.0	9.9	13.9	7.1	10.1	22.8°	37.4°	94-82
	2/6/21	6.0	1.1	2.6	6.1	1.7	3.1	19.1°	36.9°	78-68
	9/16/21	10.4	2.6	3.8	8.7	4.0	6.1	19.8°	36.5°	64
J. L.	26/5/21	10.8	4.4	7.3	10.6	5.4	7.3	18.8°	36.9°	90-80
	31/5/21	16.4	14.0	13.9	12.1	10.0	10.8	22.4°	36.8°	87
	3/6/21	14.0	6.1	9.1	9.2	4.1	6.3	20.4°	36.9°	85-80
F.	1/6/21	6.2	1.7	3.4	5.3	1.2	4.2	20.4°	36.3°	70-68
D. D. H.	30/5/21	6.9	3.1	6.3	7.5	5.2	6.1	22°	36.8°	92-88
	31/5/21	16.4	6.3	5.7	10.2	4.0	2.7	22.7°	36.9°	90
	27/5/21	5.0	3.6	3.6	5.6	4.6	4.4	19.3°	36.9°	68-66
S. U. P.	16/6/21	9.5	5.2	8.2	8.7	7.0	7.7	22.7°	36.8°	66-64
	21/6/21	6.8	5.2	5.7	7.4	6.4	5.8	22°	36.3°	66-59
	22/6/21	9.4	8.1	7.2	11.6	9.2	9.0	24.8°	36.9°	67-64
B.	2/6/21	9.3	5.0	4.3	6.1	3.1	4.0	18.7°	36.7°	66-61
C. N.	4/6/21	6.2	1.7	3.4	5.5	3.3	3.4	19°	36.8°	72-66
	16/6/21	8.0	3.5	3.1	8.0	4.5	4.6	21.3°	36.8°	78-74
C. B.	15/7/21	12.0	9.2	7.4	9.3	9.2	7.4	27°	36.9°	67-65

A = normal flow for 10".

B = electric fan on right elbow for 10".

C = fan stopped and elbow covered for 10".

an estimate of the normal flow. It is seen from this curve that the response to cold is very prompt, and the return of the flow to normal after the removal of the stimulus is equally sharp. The flow in many cases is reduced to 20 per cent or 30 per cent by the application of cold and after its removal the flow mounts again to normal or nearly so within a few minutes. In all cases readings were taken for from ten to twenty minutes subsequent to the removal of the hand from the cold water. The hand upon its removal from the water was thoroughly dried and wrapped in a towel to avoid cooling from evaporation and draughts.

(2) *Effect of the local application of hot water upon the blood-flow in the*



*hands.*—The application of heat, as by immersion of the opposite hand in hot water, produced an equally prompt response, the flow being very definitely increased (Fig. 8 and Table VI). The magnitude of the response, however, was not as great as in the employment of cold, this probably being for the reason that a greater disparity existed between the cold water and the body temperature than was the case with the use of hot water; the temperature of the latter was from 47° C. to 49° C. It appears probable from a general review of the results that the degree of the response bears a more or less direct relationship to the ratio existing between the body temperature and the applied temperature. This point, however, has not been especially investigated.

It has been found that though different normal individuals vary to some

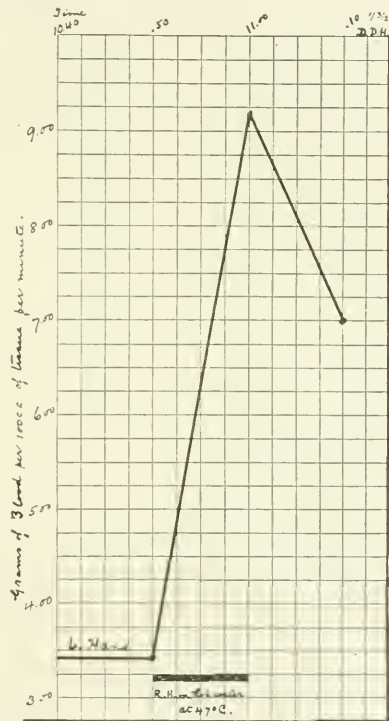


Fig. 8.—Vascular reflex to heat. Blood-flow in left hand before, during and after the immersion of the right hand in hot water.

extent in their reactions to hot and cold applications, the responses are roughly similar in all. In certain pathologic conditions, on the other hand, we have found the responses to temperature changes to be considerably below the average. A report of such cases we will reserve for a future communication.

(3) *Effect upon the blood-flow in the hands of the applications respectively of hot and cold water to the feet.*—Reflex changes in the blood-flow through the hands were also elicited by the application of extremes of temperature to the feet. One or the other foot was placed in cold water at 10° C. or in hot water at 47° to 49° C., and observations upon the flow in both hands were taken each minute for ten-minute periods. There was in every case a very

prompt response to cold, the flow dropping in one case from 7.6 gms. per 100 c.c. per minute to 1.8 gms. in the course of a ten-minute period. The normal flow was regained within a few minutes of the removal of the foot from the water. The drop as a rule was not so profound as when the cold was applied to the opposite hand. Both hands were affected alike, the curves when plotted for each running practically parallel courses (Fig. 9). In all the subjects observed the reaction was invariably a diminution of the flow in

TABLE VI  
VASCULAR REFLEX TO HEAT AND COLD STIMULUS APPLIED TO RIGHT HAND

SUBJECT	DATE	GRAMS BLOOD PER 100 C.C. OF TISSUE PER MINUTE			
		LEFT HAND			
		A	B	C	D
C.	21/12/19	6.9	3.7 ×	8.3 ×	6.7
	10/1/20	9.6	21.7	5.1	10.1
T.	5/1/20	9.5	14.4	1.6	1.6
	6/1/20	11.8	15.0	8.6	7.0
	7/1/20	3.9	9.5	6.9	4.1
	8/1/20	7.1	9.0	7.7	5.4
	8/1/20	6.6	18.2	0.7	4.2
	8/1/20	13.3	17.6	4.7	8.9
- R.	7/1/20	7.8	18.4	2.2	1.3
	8/1/20	6.6	9.3	0.1	1.2
M. D.	15/1/20	4.9	20.4	1.8	18.0
	23/1/20	6.1	9.8	2.6	7.7
	28/1/20	7.8	10.4	4.9	5.2
- P.	13/1/20	10.9	10.7	7.5	4.6

A = Max. flow before stimulus was applied.  
 B = Max. flow when heat (47° C.) was applied to right hand.  
 C = Min. flow when cold (10° C.) was applied to right hand.  
 D = Max. flow after right hand was removed.  
 ×× = right hand placed in cold water (10° C.) first.

TABLE VII  
VASCULAR REFLEX TO COLD  
BLOOD-FLOW IN HANDS WHEN FOOT WAS PLACED IN COLD WATER

SUBJECT	DATE	GRAMS OF BLOOD PER 100 C.C. OF TISSUE PER MINUTE						ROOM TEMP. °C.	MOUTH TEMP. °C.	PULSE RATE PER MIN.
		RIGHT HAND			LEFT HAND					
		A	B	C	A	B	C			
D. D. H.	10/11/20	(a) 3.3	1.9	1.1	4.0	2.3	0.9	23.2°	37.1°	74
	1/12/20	(b) 7.1	4.8	7.1	8.5	5.0	7.1	23°	37.05°	88
N. R. H.	12/11/20	(a) 7.6	1.8	4.7	6.3	1.6	5.9	21.5°	36.8°	92
R. J.	19/11/20	(a) 6.1	7.4	7.0	7.2	5.7	6.4	23°	37°	66

A = normal flow for 10".  
 B = foot placed in cold water for 10" — (a) = right foot, (b) = left foot placed in cold water.  
 C = foot removed from cold water and covered—readings taken for 10".

both hands when either foot was placed in cold water (Table VII). On the other hand when heat was applied to one foot, by its immersion in water at about 47° C., the response of the flow in the hands was not in the same direction in all individuals. Some, for instance D. D. H. and R. J., gave a prompt fall similar to the response to cold, whilst others gave a prompt rise in the flow. (Table VIII, Figs. 10 and 11.) The type of the response whether a rise or fall was always the same for the same individual. The possibility of the

drop in blood-flow evoked by hot applications, being due to cooling of the ankle consequent upon accidental wetting and increased evaporation, was guarded against with especial care, so that this may be excluded as a factor in the production of the unexpected response to heat seen in some individuals. The reduction in the flow met with in the one class of subjects was of very much the same magnitude as the augmentation of flow occurring in the other, the one type of curve being approximately the reverse of the other. The rise or fall was about the same as that following the application of cold, being from 50 per cent to 60 per cent.

Martin and Mendenhall<sup>11</sup> have shown that the vascular response to sensory stimulation is reversed when the strength of stimulus is increased above a certain threshold. A mild stimulus in the hands of these authors produced a dilatation, and a strong stimulus, a constriction of vessels. A probable explanation of the apparently contradictory results obtained by us in different individuals is that the threshold for vasoconstriction varies in different persons. Since the temperature of the water was the same in all our cases, a temperature capable of producing a vasoconstriction in one subject, might be able to bring about vasodilatation only, in another. That persons vary considerably in their sensibility to hot water is well known. A temperature unpleasantly hot to one feels tepid to another. Unfortunately no data was collected regarding the skin sensations of the subject during the immersion of the foot. We hope, however, to increase the number of these experiments when observations of this nature will be made with a view to determine whether or not, any relationship exists between the skin sensations and the vascular responses.

(4) *The effect of draught upon the blood-flow in the hands.*—Since the immersion of the hand in cold water has such a profound effect upon the flow in the hand of the opposite side as well as upon the hand of the same side, it was to be expected that lowering of the skin temperature by a current of air would likewise reduce the flow of blood through the tissues of the hands.

TABLE VIII  
VASCULAR REFLEX TO HEAT  
BLOOD-FLOW IN HANDS WHEN FOOT WAS PLACED IN HOT WATER

SUBJECT	DATE	GRAMS OF BLOOD PER 100 C.C. OF TISSUE						ROOM TEMP. °C.	MOUTH TEMP. °C.	PULSE RATE PER MIN.
		PER MINUTE								
		RIGHT HAND			LEFT HAND					
		A	B	C	A	B	C			
I G. D.	7/ 1/21	(a) 6.5	8.9	7.6	6.8	10.2	9.1	22°	36.7°	99-82
	16/11/20	(a) 0.3	1.6	2.7	1.0	1.8	3.1	21°	37.5°	112-90
D. D. H.	15/12/20	(b) 6.3	5.8	3.5	5.6	5.4	4.5	23.2°	37.2°	84-88
	19/11/20	(a) 11.1	7.2	5.6	8.5	6.8	4.2	23°	37°	88
R. J.	24/11/20	(a) 13.3	9.4	10.6	11.0	8.6	7.8	22°	36.7°	70
II N.B.T.	21/2/21				(a) 6.4	6.3	9.7	21°	36.2°	73
I. E.	25/2/21				(b) 16.0	18.3	19.8	24°	37.2°	83
S. U. P.	26/2/21				(b) 4.1	2.1	3.8	20°	36.7°	68
J. L.	4/3/21				(a) 3.0	6.2	7.4	20.1°	36.3°	94-85

A = normal flow for 10".

B = foot placed in hot water for 10"—(a) right foot; (b) left foot.

C = foot removed from water and covered while readings were taken for a further 10".

II = left hand only in calorimeter—right hand kept covered.

In order to study the precise effect of draught, experiments were carried out as follows—one arm was bared to the shoulder and covered with a blanket while the corresponding hand as well as that of the other side was placed in a calorimeter. An electric fan (18" in diameter) was placed at a distance of 3 feet from the subject. An estimation of the normal flow was made for a preliminary period of ten minutes. At the end of this time the blanket was removed and the fan started to revolve at a moderate speed so as to direct a constant current of air upon the arm for an interval of ten min-

Fig. 9.

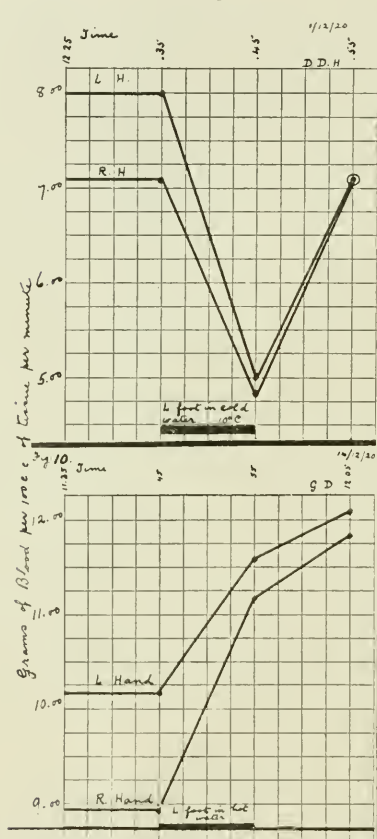


Fig. 10.

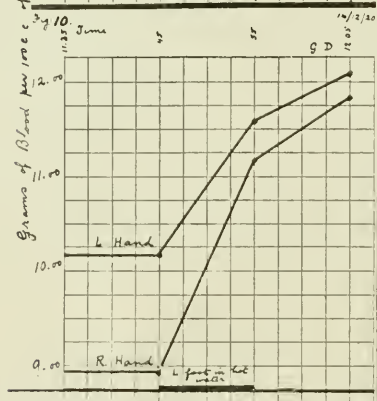


Fig. 11.

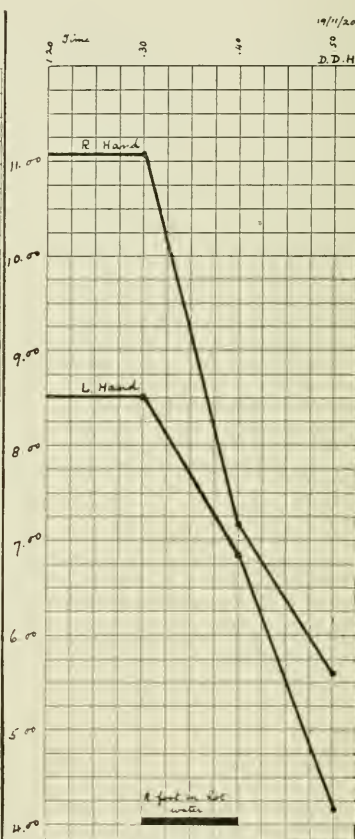


Fig. 9.—Vascular reflex in hands to cold applied to one foot.

Fig. 10.—Vascular reflex in hands to heat applied to one foot.

Fig. 11.—Vascular reflex in hands to heat applied to one foot.

utes; at the end of the time the fan was stopped, the arm re-covered and estimations of blood-flow made during a final period of ten minutes. The calorimeter was well protected from the air current so as to diminish self cooling. The flow in every instance was greatly curtailed in both hands during the time the draught was playing upon the skin. The fall produced was very nearly as great as that resulting from the immersion of the hand in cold water; a reduction of the flow by 80 per cent was common. Since draught



## BLOOD-FLOW IN MAN

TABLE IX  
VASCULAR REFLEX TO DRAUGHT

1. DRAUGHT DIRECTED TO LEFT ARM							2. DRAUGHT DIRECTED TO RIGHT ARM								
SUBJECT	DATE	GMS. BLOOD PER 100 C.C. TISSUE PER MIN.			ROOM TEMP. °C.	MOUTH TEMP. °C.	PULSE RATE PER MIN.	SUBJECT	DATE	GMS. BLOOD PER 100 C.C. TISSUE PER MIN.			ROOM TEMP. °C.	MOUTH TEMP. °C.	PULSE RATE PER MIN.
		A	B	C						A	B	C			
N. R. H.	10/2/20	× 13.4	1.9	5.1	23°	36.8	78	B. H.	8/6/20	1.2	0.7	0.3	20°	36.9°	82.73
B.	16/2/20	× 10.5	6.9	9.1	22.5°	36.4°	69	K. McM.	17/6/20	3.0	1.4	1.8	19°	37°	70
Ball	16/2/20	× 9.5	6.0	6.4	22°	36.8°	—		11/6/20	19.8	5.6	5.0	24.9°	36.8°	69
H.	16/2/20	8.0	2.4	6.1	22°	37.2°	72		15/6/20	12.3	4.9	5.7	23°	36.7°	74
N. R. H.	17/5/20	1.4	0.7	1.3	17.8°	36.9°	75	I. McG.	14/6/20	18.4	6.6	10.2	23°	36.1°	93.84
	25/5/20	5.0	1.2	2.8	19°	36.7°	76	D. H.	16/6/20	5.1	2.1	5.2	21°	36.1°	88.75
K. H.	18/5/20	4.8	0.7	2.7	18°	36.9°	74	E. M. J.	18/6/20	3.5	1.4	3.8	18°	36.7°	80
I. McG.	18/5/20	5.4	0.5	3.4	18°	36.9°	73	P.	21/5/20	8.1	3.7	4.9	21.7°	36.9°	70
	19/5/20	8.1	3.5	6.6	19.2°	36.8°	76		3/6/20	9.7	8.1	7.2	22°	36.5°	64
D. D. H.	19/5/20	1.2	0.9	3.0	19.7°	37°	84		7/6/20	3.3	1.7	2.1	19.5°	36.7°	64
E. M. J.	20/5/20	5.1	2.3	3.3	19°	37°	78		10/6/20	11.4	5.9	6.4	22°	36.8°	66
J. H.	21/5/20	1.9	0	2.1	19.7°	37°	57-72		15/6/20	8.8	5.7	8.3	23.5°	36.9°	64
	28/5/20	1.9	0.3	1.7	19.2°	36.7°	56-61		16/6/20	7.0	2.5	3.2	20°	36.5°	64
B. H.	2/6/20	11.3	7.4	9.4	23°	36.8°	88-61	N. R. H.	18/6/20	0.9	0.9	0.7	18°	36.9°	64
K. McM.	3/6/20	6.2	4.2	3.8	22.6°	36.8°	60		2/6/20	12.4	4.9	9.1	23.8°	37°	74
									9/6/20	7.9	2.1	2.6	20.1°	36.7°	92.84
									11/6/20	12.7	3.6	8.8	24.7°	36.9°	70

A = normal flow.  
B = during draught.  
C = after draught.  
× = elbow wet.

TABLE X  
SHOWING THE EFFECT OF EXERCISE (WITHOUT DYNAMOMETER) ON THE BLOOD-FLOW  
IN THE HANDS

SUBJECT	DATE	GRAMS OF BLOOD PER 100 C.C. OF TISSUE PER MINUTE						DURATION OF EXERCISE	
		RIGHT HAND			LEFT HAND				
		A	B	C	A	B	C		
M. R.	29/11/19	6.7	7.5	9.6	5.4	6.9	9.3	2 mins.	
	2/12/19	7.9	7.0	7.8	8.2	7.5	8.9	"	
	3/12/19	9.4	8.9	7.2	9.3	10.2	7.9	"	
	4/12/19	11.5	11.2	7.4	10.3	13.8	8.6	5 "	
	5/12/19	6.4	6.2	6.3	7.1	8.7	9.2	"	
	5/12/19	7.1	4.3	3.3	7.6	6.3	6.3	"	
	6/12/19	5.2	4.7	3.8	6.2	6.3	6.4	"	
	8/12/19	8.3	5.2	4.8	8.5	6.6	6.3	"	
	9/12/19	3.8	3.5	4.1	2.2	6.9	6.8	"	
	10/12/19	7.9	5.7	5.8	8.4	7.9	8.9	"	
	11/12/19	9.6	7.6	7.8	9.4	8.2	8.0	"	
	12/12/19	7.3	7.4	4.3	7.9	7.7	6.3	"	
	12/12/19	6.0	3.5	3.5	3.9	4.4	5.6	"	
	13/12/19	7.7	6.0	3.3	8.7	8.8	7.8	"	
	L.	29/11/19	8.1	7.9	7.0	8.2	9.7	8.1	"
				6.3x			6.9x		
L.	1/12/19	7.3	5.2	4.9	9.9	9.9	7.9	2 "	
	2/12/19	7.8	8.8	5.9	11.8	11.2	8.0	"	
	3/12/19	10.1	7.1	5.7	12.1	11.6	8.6	"	
	4/12/19	3.5	5.0	5.0	7.1	8.8	6.7	5 "	
	5/12/19	8.9	7.3	7.4	11.2	9.2	9.3	"	
	B.	4/12/19	7.2	—	8.5	10.4	—	6.8	2 "
	8/12/19	3.9	5.5	4.9	7.0	9.4	8.3	5 "	
	N. B. T.	9/12/19	10.9	13.4	12.8	10.8	14.8	11.8	"
		10/12/19	11.1	11.3	10.3	10.8	11	11.1	"
		11/12/19	12.2	13.4	12.3	6.1	11.9	9.1	"
12/12/19		13.1	11.5	8.6	11.3	16.1	8.5	"	

A = normal flow for 5".

B = flow when left hand was exercised 2" or 5".

C = after exercise was stopped—readings taken for 5".

X = Readings taken for 10" after exercise was stopped.

produced much less depression of temperature of the part than did immersion of the hand in cold water, the arm feeling only faintly cool under the influence of the air current, the drop in the blood-flow curve in the case of draught indicates a much greater relative response to cooling by this agent than to the direct application of cold water. The greater response in the case of draught is probably accounted for by the greater skin area influenced by the temperature change and consequently the greater number of receptors involved (cf. Martin and Stiles<sup>12</sup>). Fig. 7 exhibits the profound effect upon the blood-flow. (See also Tables V and IX.) As a rule the depression is followed by a sharp recovery upon removal of the draught. No observations were undertaken to determine the effects of draught directed to more remote parts of the body such as the back or feet.

In connection with the foregoing observations upon draught the work of Mudd and Grant<sup>8</sup> should be mentioned. These observers studied the effects of chilling of the body surface, upon the vascularity of the mucous membranes of the nasopharynx and remote unchilled areas of the skin. Changes in temperature of the skin and mucosa were recorded by means of thermopiles. Their results accord with our own; chilling of the skin surfaces by draught

produced, contrary to the general conception of the reactions occurring in such instances, depression of temperature and vasoconstriction of the mucous membranes. Distant unchilled skin areas, e.g. scars, also responded by a fall in temperature.

(5) *Observations made to determine the effect of local exercise upon the blood-flow in the hands.*—Some 50 experiments of this character were undertaken. Each hand according to the usual procedure was placed in a calorimeter and readings taken for a preliminary period of ten minutes. One hand while still

retained in the calorimeter was then exercised for five minutes, during which time and for ten minutes afterwards, readings upon the two hands were continued. Opening and closing of the hand at the rate of 30 to 40 complete movements per minute was the form of exercise employed. In one group of experiments the hand was merely opened and closed at this rate without performing work; in a second series the hand was made to work against the resistance of a stiff spring consisting of a flat band of tempered steel  $\frac{1}{32}$ " in thickness,  $\frac{3}{4}$ " wide, and 6" long, bent into the form of a bow and held in this position by means of a leather thong representing the bow-string. For the compression of such a dynamometer, so that the extremities of the steel band were approximated, 100 gram-meters of work were required.

The blood-flow through the hand almost invariably showed a decided reduction when the opposite hand was exercised and tended to regain its original level when exercise ceased. The flow in the exercised hand rose in a slightly preponderating number of cases. In the remaining cases it fell (Tables X and XI). We were unable at any time to predict which effect would ensue, for the same individual under apparently the same conditions would at one time show a rise, and at other times a fall in the flow through this hand. The effect upon the contralateral hand was unequivocal, a decided fall being the rule in all the individuals examined. The curves in Fig. 12 show an augmented effect

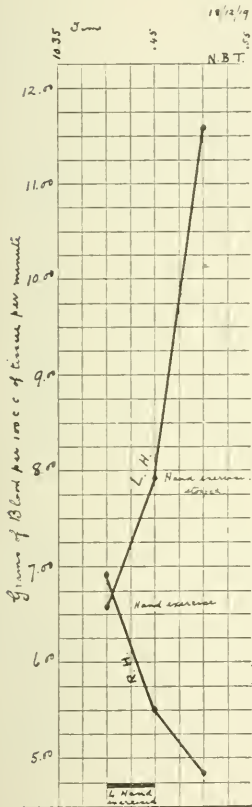


Fig. 12.—Effect of exercise. Blood-flow in both hands during opening and closing movements of left hand.

in the exercised, and a reduction of the flow in the opposite hand.

These results are contrary to those recorded by Hewlett<sup>9</sup> by the plethysmographic method, who states that exercise of one arm has little effect upon the flow in the other. When an effect was produced it was one of augmentation. Stewart,<sup>10</sup> however, found a moderate diminution in the flow of the contralateral hand.

No observations have been made regarding the effects of general body exercise upon the flow.

TABLE XI

THE EFFECT OF EXERCISE WITH DYNAMOMETER ON THE BLOOD-FLOW IN THE HANDS

SUBJECT	DATE	GRAMS OF BLOOD PER 100 C.C. OF TISSUE PER MINUTE						ROOM TEMP. °C.	MOUTH TEMP. °C.	
		RIGHT HAND			LEFT HAND					
		A	B	C	A	B	C			
I N. B. T.	15/12/19	5"	5"	5"	5"	5"	5"			
		8.3	3.4	3.4	9.1	6.6	8.3			
	16/12/19	8.1	6.3	6.5	7.6	7.7	10.8			
	16/12/19	6.2	5.7	4.5	8.8	9.4	12.6			
	17/12/19	6.9	4.6	4.6	6.4	4.8	8.0			
	17/12/19	5.2	4.6	5.8	4.9	4.4	8.6			
	18/12/19	5.9	6.3	5.0	5.5	8.1	11.4			
	21/12/19				6.7	10.5	9.8			
	23/12/19				2.2	5.2	6.4			
	29/12/19				8.6	9.2	10.6			
	29/12/19				3.4	5.0	6.2			
	29/12/19				4.6	7.1	6.2			
	II S. U. P.		10"		10"	10"		10"		
29/ 9/20		5.0	4.0	7.0	5.2	4.9	6.7	19.2°	36.8°	
30/ 9/20		2.8	2.4	1.9	3.9	2.9	2.0	16.5°	36.7°	
1/10/20		0.9	1.2	2.1	0.5	0.3	0.3	15°	36.8°	
2/10/20		1.9	2.1	2.0	0.9	0	0.7	15°	36.6°	
8/10/20		3.5	1.6	1.5	4.4	1.7	0.7	21°	36.8°	
13/10/20		5.9	5.2	5.3	8.1	7.3	7.0	20.5°	37.1°	
D. D. H.		29/9/20	1.6	1.3	2.9	1.3	0	0.8	20°	36.9°
		6/10/20	0	2.4	2.7	0.7	1.2	0.8	19°	36.9°
		15/10/20	5.4	3.1	3.5	6.9	3.2	4.2	22.5°	37.3°
McC.		5/10/20	4.2	3.5	4.2	5.4	3.8	3.8	17.3°	36.7°
		7/10/20	3.7	3.2	2.6	4.3	0	1.9	19°	36.9°
		11/10/20	6.7	6.3	6.9	6.4	6.4	6.5	20°	36.9°

A = normal flow.

B = I left hand exercised.  
B = II right hand exercised.

C = exercise stopped and hand resting.

## SUMMARY

1. Certain minor modifications in the calorimetric method of blood-flow estimations (Stewart's) are described.

2. From a large number of blood-flow determinations upon normal individuals it has been found that the amplitude of the flow differs widely in different persons; that the flow fluctuates spontaneously to the extent of several grams per 100 c.c. per minute in the same individual during the course of an experiment; and that marked alterations in the flow are effected by changes in the temperature of the atmosphere.

3. The flow in the hand may be influenced reflexly by applications of heat or cold to either hand or foot. Thus, immersion of the hand in hot or cold water produces a rise or fall respectively in both hands. The response to draughts is similar in nature to the response following the immersion of the hand in cold water. Heat applied to the feet produces in some individuals a rise and in others a fall in the blood-flow through the hands; the particular effect produced is constant for the same individuals.

4. Local exercise produces a drop in flow of the opposite hand. The flow in the exercised hand is increased in some cases and reduced in others, the effect being unpredictable.

The investigations embodied in this paper were suggested by Professor J. J. R. Macleod and carried out under his direction. For his interest, encour-



agement and kindly criticisms the author feels sincerely grateful. The author also wishes to express his appreciation of the assistance of Miss N. R. Hearn who carried out a great number of the blood-flow estimations and prepared to a large extent the tables and curves shown in this paper.

Funds for this research were furnished by the Medical Research Committee of the Department of Militia and Defence of Canada.

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## PANCREATIC EXTRACTS\*

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IN a previous paper<sup>1</sup> we have reported experiments which justify the conclusion that some constituent of the pancreas destroys the active principle of the internal secretion of the gland when extracts are made of the gland by the usual methods. To eliminate these digestive substances, extracts were prepared from degenerated pancreatic tissue ten weeks after ligation of the ducts of the pancreas by which time the acinar but not the insular cells are said to have disappeared. From this material we secured small quantities of very active extract. The question of a more rapid and economical method of securing larger quantities of the extract soon became of prime importance.

Ibrahim<sup>2</sup> could obtain no conclusive evidence of the presence of an active proteolytic enzyme in the pancreas of the human fetus till after the fourth month of intrauterine life. Carlson<sup>3</sup> reported that in pregnant bitches near term, complete pancreatectomy was not followed by severe glycosuria in uncomplicated cases till the young were born. Allen<sup>4</sup> was unable to confirm this finding. The most natural interpretation of Carlson's result is that the pancreas of the fetus furnishes to the mother an internal secretion which is necessary for the metabolism of sugar. These facts coupled with the evidence afforded by our previous experiments suggested the possibility that the fetal pancreas might prove a source of an extract rich in internal secretion and yet free from the destructive enzymes of pancreatic juice.

In order to test this hypothesis a quantity of pancreas was obtained from fetal calves of less than five months' development. The tissue was macerated in Ringer's solution, and the liquid filtered off. The filtrate was tested on several different diabetic dogs and found to produce similar effects upon the percentage sugar of the blood and on the sugar excreted in the urine as did the extract prepared from degenerated pancreatic tissue. The extract was not found to contain any proteolytic enzyme.

In this paper we are reporting two experiments in which such extracts of fetal calf pancreas were used.

### EXPERIMENT I

A total pancreatectomy was performed upon Dog 27 on November 14; weight of the animal, 5 kgm. The effects of injections of the extract are given in Table I.

### EXPERIMENT II

Chart I is the graphic record of some interesting experiments performed upon a dog (No. 33) from which the pancreas was removed on November 18,

\*From the Department of Physiology, University of Toronto.

TABLE I  
Dog 27—PANCREATECTOMY NOVEMBER 14—WEIGHT 5 KILOGRAMS

DATE	HOUR		BLOOD SUGAR	EXTRACT	SUGAR EXCRETED	WEIGHT
Nov. 15	10	A. M.	.11	-	-	5.0 k. (Total pan- createctomy)
Nov. 14	3	P. M.	.28	-	-	-
Nov. 17	8.30	A. M.	.30	5 c.c.	12 noon 16 to	4.1 k.
	9.15	A. M.	.20	-	9 A. M. 17 -	
	10	A. M.	.17	5 c.c.	4 gms.	
	6	P. M.	.15	5 c.c.		
Nov. 18	9	A. M.	.175	10 c.c.	Previous 24 hr.	4.1 k.
	10	A. M.	.08	-	vol. of urine	
	6	P. M.	-	10 c.c.	100 c.c., no sugar.	
Nov. 19	9	A. M.	.21	-	Previous 24 hr.	4.2 k.
	6	P. M.	-	10 c.c.	vol. of urine 225 c.c. sugar free	
Nov. 20	10	A. M.	.20	10 c.c.		4.3 k.
Nov. 21	10.15	A. M.	.26	10 c.c.		
Nov. 22	9	A. M.	.25	12 c.c.		
Nov. 23	9	A. M.	-	12 c.c.		
Nov. 24	9	A. M.	-	10 c.c.		
Nov. 25	9	A. M.	-	10 c.c.		
Nov. 28	6	P. M.	-	2 c.c. (concentrated)		
Nov. 29	6	P. M.	-	5 c.c. (concentrated)		
Dec. 2	11	A. M.	.15	-	Previous 24 hr.	4.0 k.
	12	A. M.	-	6 c.c.	vol. 320 c.c. .5 gms. sugar.	
Dec. 4	12	A. M.	-	6 c.c.		

Dog 27 was removed from the metabolism cage on November 20. Sugar excretion was not followed in the interval between November 20 and December 1. On November 20 the animal was in excellent condition which was maintained until December 2. At noon December 2, six c.c. of fetal calf extract prepared 16 days previously was injected subcutaneously. This extract had been prepared under aseptic conditions and immediately after its preparation was found to have a very low bacterial count. The solution was kept in a refrigerator some distance away from the ice. At 3:30 P. M. December 2, the dog showed symptoms of a peculiar nature. Periods of unconsciousness with convulsive twitchings, retraction of the head, salivation and frothing alternated with periods of semi-consciousness. These symptoms lasted from 3:30 P. M. to 6 P. M., after 6 P. M. the animal gradually improved and at 8 P. M. was resting quietly. On December 4 at 12 A. M., 6 c.c. of the same extract was again given. At 1:30 P. M. the animal began to exhibit symptoms similar to but more severe than those noticed on December 2. The dog died during the night of December 4. Postmortem examination showed that all the pancreas had been removed and there was no evidence of infection. Twelve c.c. of the same extract as was used in the latter part of this experiment injected into a normal dog produced no apparent reaction.

1921. Daily sugar excretion in grams and 24 hour urine volume are clearly shown in the chart, from which it will be seen that in twenty-four hours after the operation (November 19, 10 A.M.), the percentage of sugar in the blood was 0.33 per cent. Ten c.c. of filtered fetal calf extract were injected intravenously and one hour later the blood sugar was 0.17 per cent. At 7 P.M. November 19, the blood sugar was 0.37 per cent when 10 c.c. of extract that had been passed through a Berkefeld filter were injected. At 8 P.M. the blood sugar was 0.26 per cent. At 9 A.M. November 20 the blood sugar was 0.37 per cent when ten c.c. of neutral Berkefeld extract which had been kept in a water-bath at 78° C. for thirty minutes were injected intravenously without causing any change in blood sugar. From this and other experiments we believe that the active principle of this extract is destroyed by heating it to

approximately 65° C. At 4 P.M. November 20, 10 c.c. of extract which had been boiled twenty minutes in the presence of 2 per cent HCl was injected intravenously. No reduction in the percentage of blood sugar was observed. Similar results were obtained with corresponding concentrations of other acids. At 7 P.M. November 20, when the blood sugar was 0.40, 10 c.c. of neutral and Berkefeld extract, given intravenously, lowered it to 0.23 per cent in three hours.

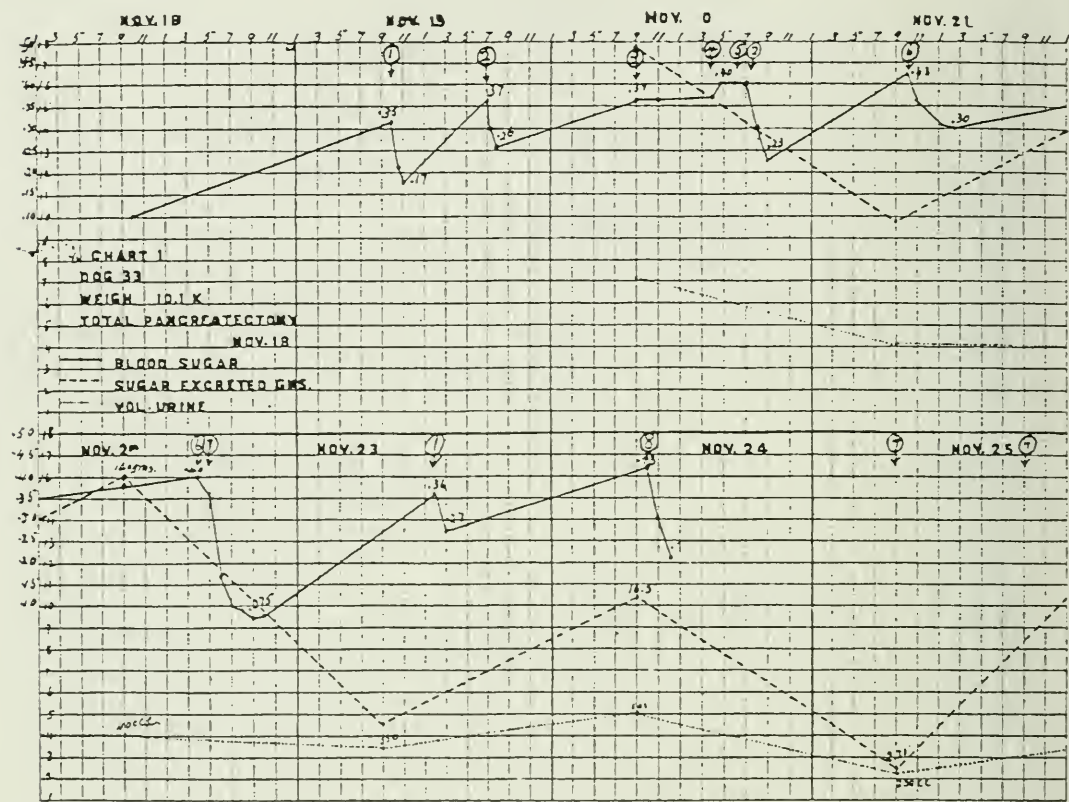


Chart 1.—(1) 10 c.c. filtered fetal calf extract intravenously. (2) 10 c.c. Berkefelded fetal calf extract intravenously. (3) 10 c.c. of (2) heated to 78° C for 30 minutes. (4) 10 c.c. of (2) + ¼ c.c. glacial acetic boiled for 30 minutes. (5) 10 c.c. of (2) + ½ c.c. hydrochloric boiled for 30 minutes. (6) 20 c.c. Berkefelded fetal calf extract subcutaneously. (7) 10 c.c. of (1) subcutaneously. (8) 10 c.c. degenerated pancreas of dog intravenously.

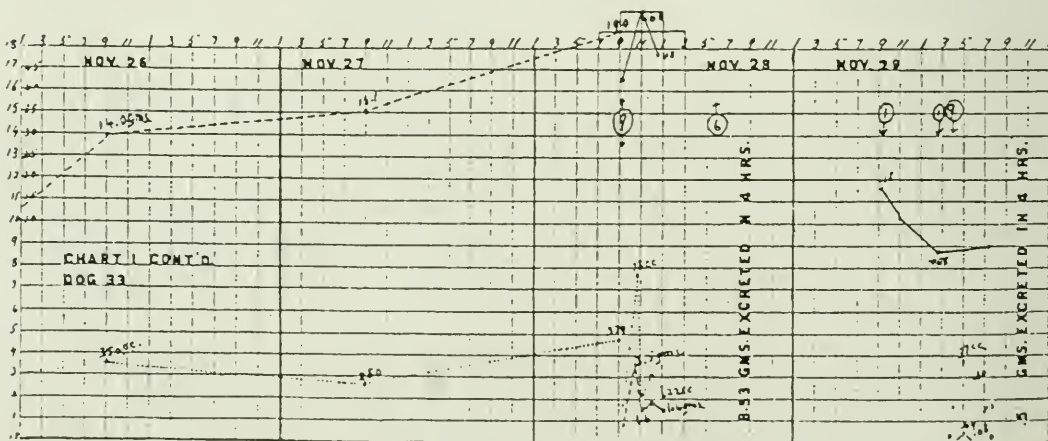


Chart 1 (continued).—(9) 100 c.c. water. 10 gms. dextrose. Dog converted to longevity experiment December 5. Chloroformed January 27, 1922.



On November 21 the blood sugar was lowered from 0.43 per cent at 10:15 A.M. to 0.3 per cent at 2 P.M. by the subcutaneous injection of 20 c.c. of Berkefeld extract. On November 22, 10 c.c. of a freshly prepared concentrated extract of fetal calf pancreas was injected subcutaneously with the remarkable result that the sugar of the blood fell from 0.40 per cent at 2 P.M. to the subnormal level of 0.075 per cent at 9 P.M. On November 24 at 10 P.M., 10 c.c. of a seven days' old extract containing 0.7 per cent tricresol caused the percentage of blood sugar to fall from 0.43 to 0.22 per cent at 11:15 A.M. On November 28 at 9:30 A.M. the blood sugar was 0.43 per cent and 10 gm. sugar in 100 c.c. of water injected intravenously raised it to 0.60 per cent and 8.53 gm. of sugar were excreted in the urine in the four hours following the injection. We were unable to follow the blood sugar curve as closely as desirable owing to the difficulty of obtaining blood by venepuncture. At 6 P.M. on the same day 6 c.c. of a more concentrated fetal calf extract were given subcutaneously and next morning (at 9:30 A.M. November 29), the blood sugar was found to be 0.18 per cent when 4 c.c. of the same extract was again injected. The blood sugar at 2:30 P.M. was 0.048 per cent and the urine collected from 9:30 A.M. to 3 P.M. was sugar free, the volume excreted between 12 noon and 3 P.M. being 68 c.c. At 3 P.M., 4 c.c. extract was administered and at 4 P.M. 10 gm. of sugar in 100 c.c. of water were injected intravenously. The blood sugar at 7:30 P.M. was 0.05 per cent and in the two hours following the injection of sugar 0.51 gm. were recovered in the urine after which the urine was free of sugar. On November 30 the animal was in good condition. On December 1 it excreted 14.84 grams of sugar in a volume of 520 c.c. of urine and on December 2, 320 c.c. of urine and 10.94 gm. of sugar.

At 10 A.M. on December 3 after eighteen hours' starvation the blood sugar was 0.28 per cent when 10 c.c. of concentrated fetal calf extract were given by stomach tube in order to find out whether absorption of the active principle of the extract might occur through the gastric mucosa. At 2 P.M. the blood sugar stood at 0.23 per cent when another 10 c.c. of extract were given by mouth. The blood sugar rose slightly during the next hour. On the following days December 4, 5 and 6 the animal excreted 4.95, 8.40 and 15.70 grams of sugar respectively.

Since the animal at this stage was still in fairly good condition, even though there had been considerable irregularity in the administration of extract, it was decided to discontinue using it for the purpose of testing the relative potency of different forms of extract and to administer to it the most efficient of these in regular dosage so as to determine for how long a time the animal could be kept alive. On December 7 the injection was made with fetal calf extract prepared as described above, but on December 8 an extract, prepared by extracting the pancreas with alcohol, evaporating to dryness and redissolving the residue in distilled water, was used with the result that 4 c.c. caused the blood sugar to fall from 0.30 to 0.15 per cent in one hour. A second injection of this extract (12 c.c.) was given at 1 P.M. and at 5 P.M. the blood sugar was 0.12 per cent. It will be seen that the principle upon

which the preparation of this latter extract depends is the same as that of E. L. Scott<sup>2</sup> and the favorable results led us to see whether adult pancreas could be used in place of fetal. Six c.c. of whole gland extract prepared as above were therefore injected daily from December 8 to January 3 inclusive. On January 4 the administration of extract was discontinued and on this day the dog excreted 12.86 grams of sugar in a volume of 450 c.c. of urine. The animal was in good condition and was on a diet of lean meat. On January 5 it was not so hungry or so lively as on previous days and 4 grams of sugar in 350 c.c. of urine were excreted. On the next day (January 6) 3.5 grams of sugar and 320 c.c. of urine were excreted and the animal was in a very poor condition compared with that of a few days previously. On January 6 the diet was changed to one of milk and biscuit along with meat, and the sugar excretion rose to 25 gm. At noon on January 7, while on the same diet, 8 c.c. of whole gland extract were injected and the twenty-four hours' sugar excre-

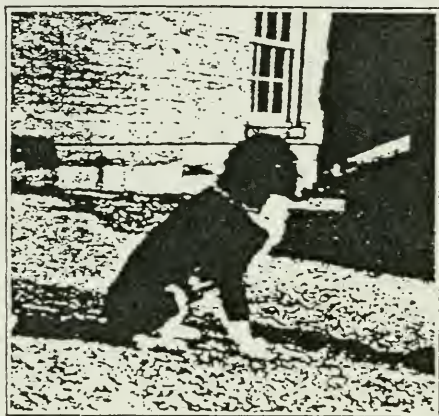


Fig. 1.



Fig. 2.

Figs. 1 and 2.—Dog No. 33 nine weeks after total pancreatectomy.

tion was found to be 22 gm. A further injection of 10 c.c. of extract at 9 A. M. on January 8 brought the sugar excretion down to 2.0 gm.

The original weight of the animal (33) was 10.1 kgm., and one week after the pancreatectomy it was 8.1 kgm. This was maintained fairly constant throughout the second and third weeks after the operation and then slightly increased. From the fifth to the ninth week (see Figs. 1 and 2), during which time daily injections of 6 c.c. of whole gland extract were given, the condition remained good and was so on the 63rd day when, to convince ourselves that the extract was necessary to the health of the animal, we discontinued its administration for three days (21, 22 or 23 of January) with the result that the dog became so weak that it was barely able to stand. Extract was again given on the 24 and 25 of January with decided improvement. On January 27 when the dog weighed 7.9 kgm. it was killed by an overdose of chloroform and a careful autopsy immediately made by Dr. W. L. Robinson, Pathologist at the General Hospital, Toronto. His report is as follows:

We made a very careful examination of this dog with the object of determining if any pancreatic tissue had been left from the operation, or if possible, an accessory pancreas were present.

The area formerly occupied by the pancreas showed no gross evidence of pancreatic tissue. There were a number of firm fibrous adhesions about the duodenum. These were sectioned, and on microscopic examination showed no evidence of pancreatic tissue remaining in them.

The duodenum was then examined and nothing abnormal found except for a small nodule (see Fig. 3) about 3 mm. in diameter situated in the wall at the mesenteric attachment and 10 cms. below the pylorus. This on micro-

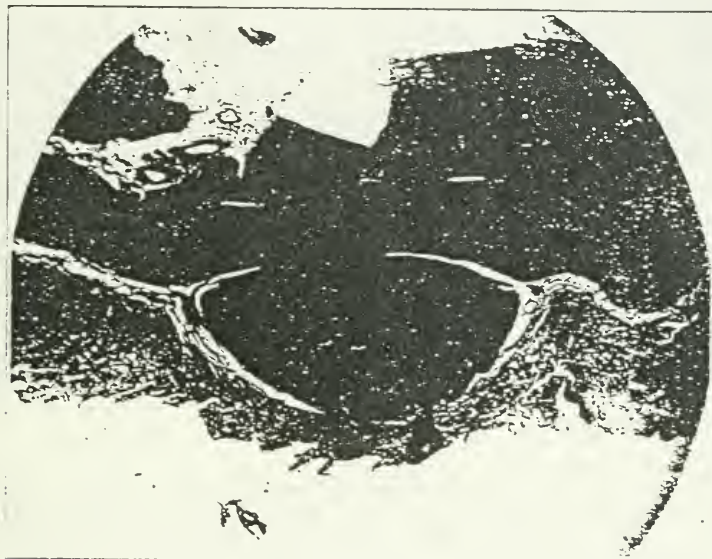


Fig. 3.—Section of wall of duodenum at seat of pancreatectomy.

scopic examination was found to consist of what is apparently a nodule of pancreatic tissue, lying in the submucosa. Serial microscopic sections of this however, failed to show the presence of any Islands of Langerhans.

No other gross or microscopical evidences of pancreatic tissue could be found.

(s) W. L. Robinson,  
Pathologist,  
Toronto General Hospital.

### EXPERIMENT III

To study the effect of an intravenous injection of whole gland (normal beef pancreas) extract upon the blood pressure of a diabetic animal. Dog 27 was anesthetized and its blood pressure recorded. (See Fig 4.) After the intravenous injection of five c.c. of extract, the blood pressure fell approximately 50 mm., but regained its original level in less than two minutes. The effect of a similar dose of extract upon the blood sugar of an unanesthetized diabetic animal would have lasted for at least six hours, and the lowest



level would have been reached in from one to two hours after the injection. The fact that administration of extract to this animal caused very little fall in the blood sugar, indicates that in anesthetized diabetic animals, extract of known activity causes only a slight reduction in the blood sugar, a fact which we had observed before. Further details of the interrelationship of the effects of anesthesia and extract will be reported later.

#### EXPERIMENT IV

The purpose of reporting this experiment is to show the relative effects of extracts prepared in various ways. (No attempt was made to prove the

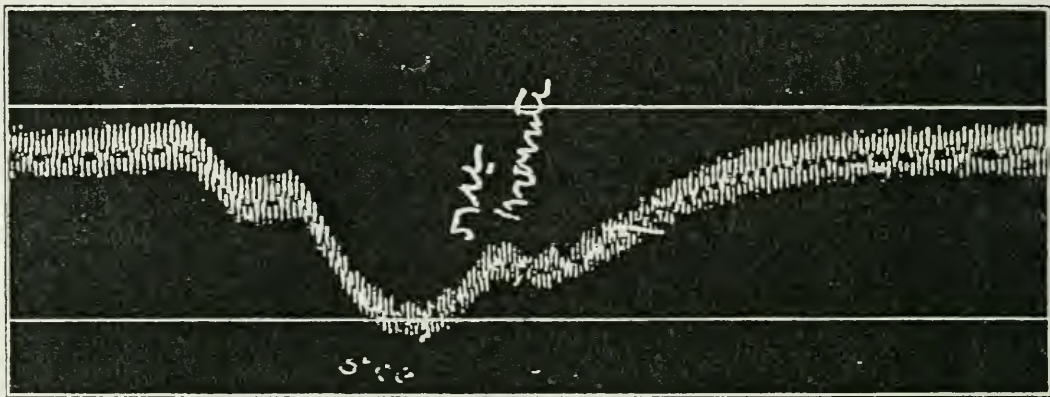
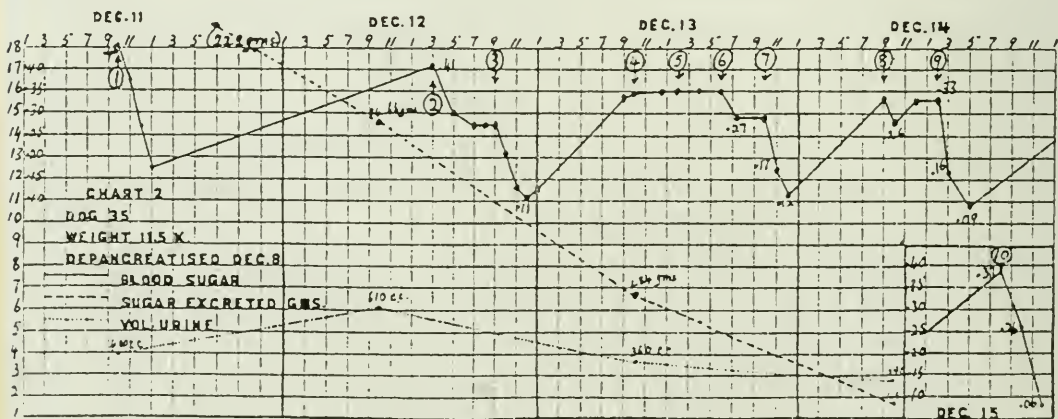


Fig. 4.—Blood pressure tracing following administration of extract.

dog totally diabetic, nor was the excretion of sugar followed closely since we wished simply to ascertain by the blood sugar whether a particular extract contained the active principle or not). On December 8, 1921, Dog 35 (see Chart II) was completely depancreatized. The animal made a good post-operative recovery. On December 11 we injected 6 c.c. of an extract prepared from the pancreas of this animal in the following way. The entire pancreas immediately after removal was cut into small pieces which were put into





0.2 per cent HCl in 95 per cent alcohol and allowed to stand till December 10. It was then macerated, filtered, and the clear filtrate evaporated to dryness in a warm air current. On December 11 this dry resin-like residue was emulsified in 25 c.c. Ringer's solution and 6 c.c. were given intravenously at 10 A.M. The blood sugar dropped from 0.46 to 0.18 per cent in three hours.

On December 12 at 3 P.M., 20 c.c. of an extract made from the pancreas of a cow in the same manner as outlined above, was given on an empty stomach by stomach tube. The blood sugar fell from 0.41 at 3 P. M. to 0.28 at 7 P. M. At 9 P. M., 10 c.c. of this same extract was given intravenously. The blood sugar fell from 0.28 at 9 P.M. to 0.11 at 12 P.M. On December 13 (see Chart II), about 20 gm. each of liver (4), spleen (5), thyroid (6), and pancreas (7) were extracted in identically the same manner as outlined above. There was no reduction in blood sugar following the intravenous injection of extracts of liver or spleen; a slight fall occurred (from 0.35 to 0.27) following the administration of extract of thyroid and a marked one (from 0.27 to 0.12) following one of pancreas.

On December 14 at 9:30, 10 c.c. of extract of thymus (8) made in the same way as were the liver, spleen and thyroid, were injected intravenously. There was a slight temporary fall from 0.33 to 0.26 in one hour. At 2 P.M., 6 c.c. of an extract prepared as above from pancreas and which had been placed in a parchment dialyzer in running water for twelve hours caused the percentage of blood sugar to fall from 0.33 to 0.09 per cent in three hours.

On December 15 200 mg. of the residue of ox pancreas extract were washed twice in toluol and then in 95 per cent alcohol, then dried again and emulsified in saline. At 10 A.M. this was given intravenously. The blood sugar dropped from 0.37 to 0.06 per cent in four hours. On December 16 at 9 A.M., 10 c.c. of concentrated fetal calf extract were given *per os*. Blood sugar fell very slightly (0.38 to 0.35 per cent). On December 19, the animal, by this time considerably emaciated, was killed by chloroform.

#### DISCUSSION

The foregoing observations were undertaken, partly to determine whether extracts having an antidiabetic power equal to those prepared from the degenerated pancreas could also be prepared from the normal gland, and partly to find out whether frequent injection with active extracts would prolong the life of a depancreated animal far beyond the limit of time which such animals ordinarily survive. In connection with the preparation of the extract it has been found that fetal ox pancreas and, with certain modifications in the method of preparation, adult pancreas as well, furnish highly potent extracts. These extracts are however somewhat toxic, and they are apt to cause local abscesses at the point of injection. This method of preparation is being materially improved by various important modifications which are being worked out by Dr. J. B. Collip and which will be reported in the near future. In connection with the effect of the extract on the longevity of the depancreated animal, one of our observations would seem to afford fairly conclusive results. This animal (33) lived for seventy days and at autopsy no

pancreatic residues were found by macroscopic examination although by microscopic examination of serial sections of the duodenum a small nodule of pancreatic tissue (containing no islets) was found in the submucosa. It does not seem likely that so small a piece of pancreas could be responsible for the maintenance of life in the animal but, of course, the experiment is not finally conclusive. We are engaged in its repetition and will report the results in due time.

#### CONCLUSIONS

By intravenous and subcutaneous injections of neutral saline extracts prepared from the pancreas of the bovine fetus at about the fifth month, the percentage of blood sugar and the daily urinary excretion of sugar are markedly reduced in depancreatized dogs.

Daily injections of extract of pancreas enabled a depancreatized dog to live for seventy days.

The active (antidiabetic) principle of such extracts is destroyed by boiling in strongly acid reaction but it is not affected by the presence of tricresol which may therefore be used as a preservative.

The depressor action of the extract is short-lived.

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## YEAST THERAPY AND URIC ACID EXCRETION\*

BY ARTHUR H. SMITH, PH.D., WITH THE COLLABORATION OF HARRY J. DEUEL, JR.,  
LEAH ASCHAM, AND FLORENCE B. SEIBERT, NEW HAVEN, CONN.

AT PRESENT bakers' yeast is being widely recommended and used as a therapeutic agent for treatment of various ill-defined irregularities of metabolism. The familiar cake of compressed yeast represents a mass of living cells containing nucleoproteins which are purine-yielding precursors of uric acid. It is also conceivable that yeast may influence endogenous uric acid formation through an effect on cellular metabolism. Carefully controlled experiments dealing with the effect of yeast ingestion on uric acid excretion have not been published. Therefore, the following studies have been undertaken to elucidate some of the questions at issue.

Salomon<sup>1</sup> has described the results of ingestion of "nutritive yeast" which is evidently a dried product used as a source of protein. He showed that, when 40, 80 and 100 grams of this substance were taken, there was a definite increase in the uric acid excretion; hence, gouty persons or those with uric acid calculi were advised to refrain from consuming large quantities of nutritive yeast. Funk, Lyle and McCaskey<sup>2</sup> likewise reported experiments on persons in whom ingestion of 20 to 60 grams of dried yeast led to an increased content of uric acid in blood and urine. The most commonly recommended dose of commercial compressed bakers' yeast is three small cakes per day representing about 12.5 grams of solids. Using the data of Meisenheimer<sup>3</sup> we estimate that the possible yield of purine nitrogen from this quantity of yeast is at most less than 0.06 grams, furnishing a negligibly small quantity of uric acid.

The uric acid excreted represents the end product of metabolism of not only the food purines but also the purines derived from body cells, i.e., it may have both endogenous and exogenous origin. Mares<sup>4</sup> suggested that the activity of glandular cells, especially those of the digestive tract, play an important rôle in the formation of uric acid. Mendel and Stehle<sup>5</sup> described experiments which seemed to bear out the idea of Mares. Work and profuse sweating have also been considered the cause of increase in uric acid output.<sup>6</sup> The question therefore arises whether the therapeutic dose of yeast, though unimportant as a source of exogenous uric acid, might prove to be a stimulus to metabolism such as would be reflected in an increase in the endogenous uric acid.

The subjects of these experiments were four persons working in the laboratory. The daily regimen was similar to the conventional "purine-free" diet based on eggs, milk and cheese, except that it was found desirable to include

\*From the Sheffield Laboratory of Physiological Chemistry Yale University, New Haven, Connecticut.  
Received for publication, November 2, 1921.

vegetable salad in the noon meal. The same kinds of food were eaten every day, and although they were not weighed it may be inferred from the total nitrogen excreted that the intake was fairly constant. The diet was low in urines. Approximately forty calories per kilo of body weight were provided, and the weight of the subjects remained constant throughout.

The fresh cakes of compressed yeast were eaten before meals as recommended in the advertisements. It is stated that for those troubled with ex-



Chart 1.

cessive gas formation after eating yeast, suspending the material in boiling water kills the cells and prevents undue fermentation. None of the persons found this procedure necessary although when large numbers of yeast cakes were eaten, there was considerable gas formation in the gastroenteric tract.

The uric acid in the urine was determined by the colorimetric method of Jolin and Wu. Hydrogen-ion concentration was ascertained by the colori-

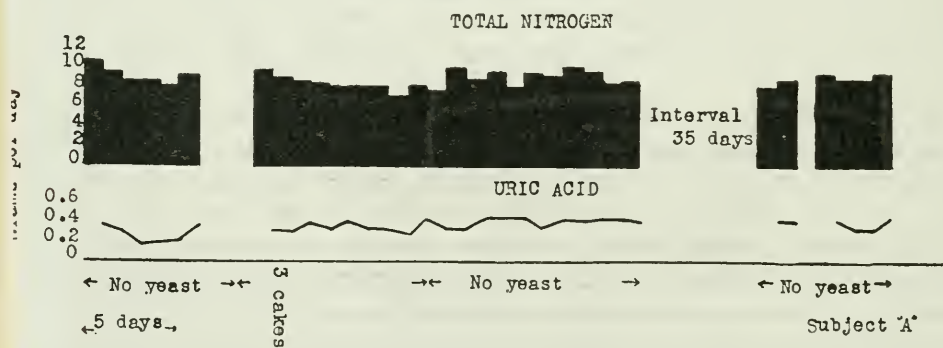


Chart 2.

etric method using methyl red and thymol blue as indicators. Total nitrogen as determined by the Kjeldahl method.

Subject "A" ate three cakes per day for ten days; subject "F" ate three cakes daily for six days and then six cakes for four days; subject "S" ate three cakes for three days, six cakes for three days and nine cakes for four days; subject "D" ate three cakes for three days, six cakes for three days, nine cakes for three days and fifteen cakes on the last day. We thus obtained



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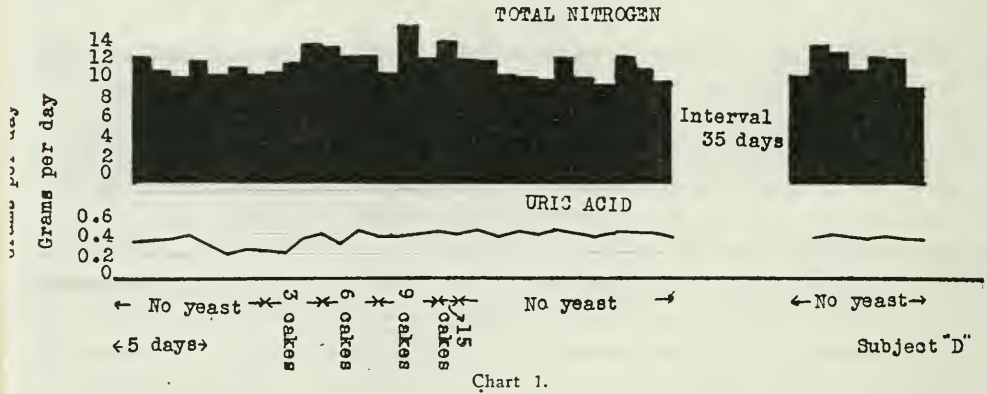
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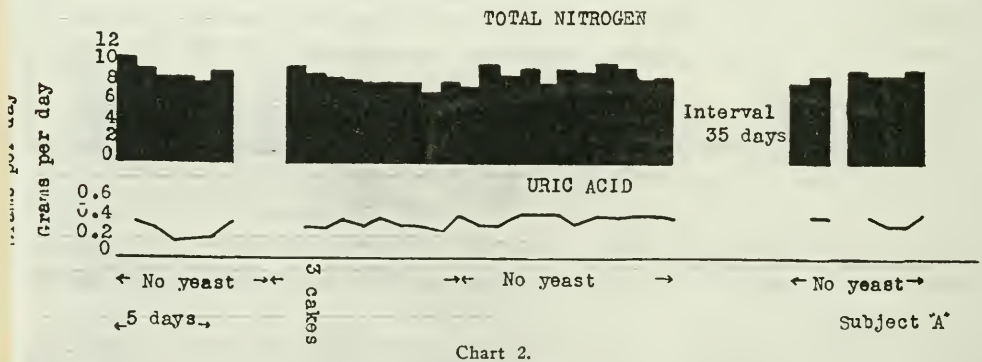
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data on the effect of the recommended dose and on twice, three times and five times that amount.

There was no significant change in the  $p_H$  of the urine of any of the subjects during the entire experiment. A mild laxative effect was noted during the yeast period, followed by some constipation during the after period. The highly digestible character of the diet favored constipation, however. The first period of the experiment extended from June 13, 1921, to July 11; then to determine whether or not there was a long delayed effect of the yeast, another control period was run throughout the week of August 15. Subject "F" had eaten yeast from the end of the first experiment up to within thirteen days of the beginning of the second control period.

As will be seen from the charts, there was no significant change in the values for uric acid elimination in any of the subjects throughout the experiment. The values in the second after-period were the same as those of the first after-period. Apparently the level of nitrogen metabolism was slightly

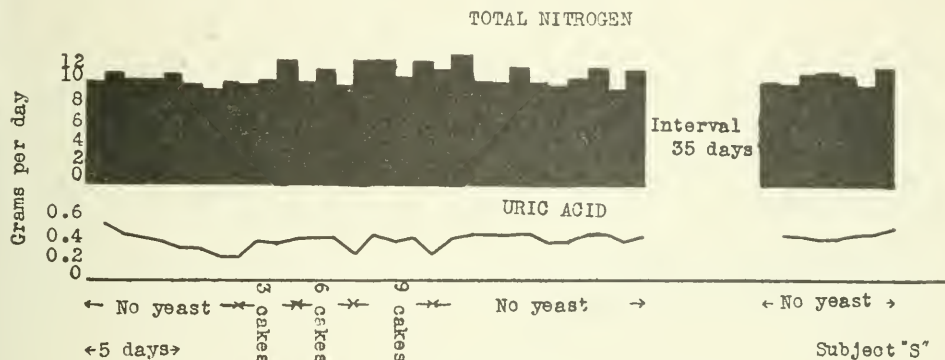


Chart 3.

higher in both after-periods which might account for the very slightly increased uric acid elimination in those periods. (See Mendel and Stehle.<sup>5</sup>) The constancy in uric acid output was maintained in spite of the fact that during certain periods three of the subjects took twice the therapeutic dose of yeast (six cakes), two ate three times the dose (nine cakes) and one ate five times the dose (fifteen cakes containing 62.5 grams of solids). That the yeast was digested to some extent is indicated by the general rise in total urinary nitrogen level during the yeast period in all but one of the subjects.

To determine whether or not yeast cells escape disintegration in any considerable number in the digestive tract, samples of feces were incubated with proper controls in the synthetic medium of Fulmer, Nelson and Sherwood.<sup>7</sup> During the yeast-feeding period viable yeast cells were found in the feces of every one of the four subjects in considerable numbers, but few cells showed budding. Unfortunately these determinations could not be carried out quantitatively; yet we feel that we were not dealing with adventitious yeast cells, for the feces of three persons not in the experiments gave negative results as did the feces of subjects "F," "D" and "S" seven days after the end of

the yeast-feeding period. It is apparent, then, that the yeast employed does not continue to grow in the human intestine even after repeated seeding.

It follows from the above data that the ingestion of live yeast is not attended with such an increase in metabolism as would augment the excretion of endogenous uric acid.

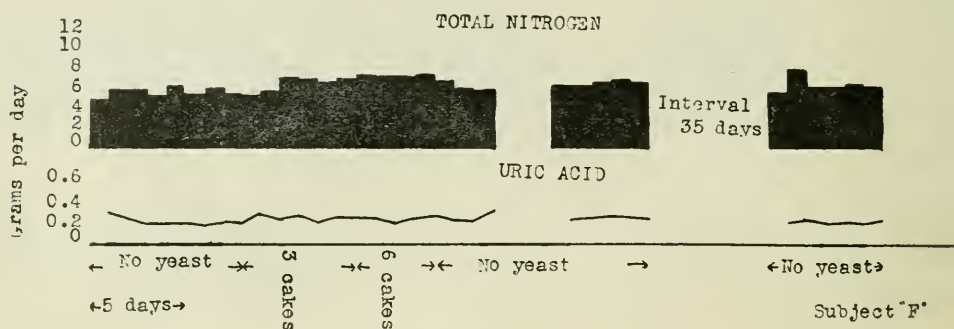


Chart 4.

## SUMMARY

There is no evidence of an increase in uric acid excretion following the ingestion of the commonly recommended therapeutic dose of live bakery yeast. When twice, three times and five times this dose was taken there was likewise no increase in uric acid excretion over the level attained on a purine-low diet.

Viable yeast cells appeared in the feces in large numbers during the yeast period but disappeared promptly after the ingestion of yeast was stopped.

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# LABORATORY METHODS

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## NEW LABORATORY APPARATUS\*

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BY JOHN L. LAIRD, M.D., PHILADELPHIA, PA.

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THE necessity for the performance of a considerable and increasing number of tests in a minimum time and with a more or less fixed staff in the Pennsylvania Department of Health Laboratories has led to the invention of a number of instruments for mechanically facilitating the work.

Their possible value and interest to laboratory men make a description of some of them and their uses seem desirable.

### AUTOMATIC PRECISION PIPETTE

This instrument was invented for the primary purpose of increasing the speed and accuracy of filling test tubes with the various reagents in the performance of the Wassermann-Bordet Reaction.

It consists of a three-way tip (Fig. 1), the base arm of which (*a*) is ground to fit a Record Syringe; the receiving arm (*b*) is supplied with a valve which is released by suction on withdrawal of the piston of the syringe and closes upon pressure on insertion of the piston; and a delivery arm (*c*) in which the valve has the opposite arrangement, closing on suction and opening upon pressure. We realize that the principles involved in this portion of the instrument are not new but it possesses the novel feature of its application and the fact that it has been successfully applied. The latter fact is largely due to the character and setting of the valves. The majority of instruments which have been similarly constructed have carried ball valves which are difficult, if not impossible, to make tight fitting; this has led to leakage which vitiates their purpose. The valves are of the hydraulic type as shown in Figure 1, which not only set firmly but are of a form which allows of the proper addition of springs which increase their rapidity of action, the importance of which will be readily seen.

The Record Syringe was chosen for the pipette because of features which render it peculiarly adaptable to the purpose: (1) All sizes of the Record Syringe fit the same tip: (2) The glass barrel allows of observance of the contents: (3) The plunger, carrying an expansion ring, fits fairly tightly and allows of very little back leakage: (4) The metal plunger, staff and cap, the staff threaded into plunger and button and the cap being firmly and easily attached, allowed of the addition of the novel adjustment which governs the quantitative receipt and delivery of the instrument. (Fig. 2.)

The adjustment consists of a metal sheath (*a*) surrounding the piston

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\*From the Division of Laboratories Pennsylvania Department of Health.  
Received for publication, Aug. 19, 1921.

staff and threaded through the syringe cap; it carries forty-eight threads to the inch allowing of nice adjustment to any position on the syringe; the plunger striking the inner end is stopped thereby at any point desired to force the receipt and delivery of a definite amount of liquid. A set-nut (*b*) fitted to the sheath may be firmly clamped down to hold the sheath in the desired position against the repeated jar of the piston.

On account of the possible positions of the sheath for varying capacities, a piston staff of double the length is substituted for the regular staff.

For automatic filling, which allows of the use of the instrument with one hand, a spiral spring is applied about the staff between the button and the top of the adjustment sheath. This requires the use of a syringe of at least

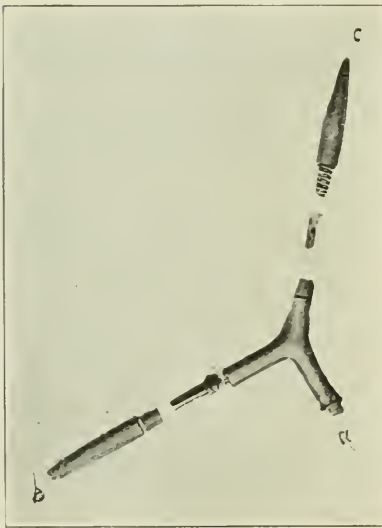


Fig. 1.

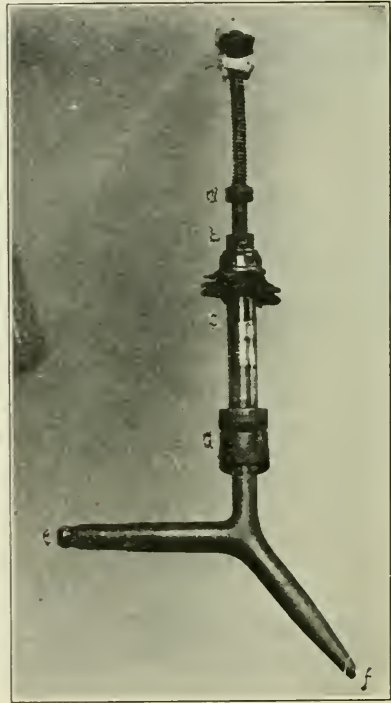


Fig. 2.

twice the capacity of the amount desired for delivery. We have found this arrangement most satisfactory for the Wassermann work. It might be added that the angle of the delivery and receiving arms (*f* and *e*) to the base of the tip as shown in Fig. 2 has also proved most convenient.

#### SETTING UP AND ADJUSTMENT

The tip is applied to the syringe precisely as one applies a needle, and held in place by either the special clamp (*d*, Fig. 2) or by means of a rubber band attached to a side-armed ring placed directly above the metal base of the syringe and extended around the receiving arm of the tip. A 1000 c.c. separatory funnel of the pear shape (*b*, Fig. 3) proved most satisfactory as a

receptacle for holding the stock solution. This is attached to the receiving arm of the pipette by a seamless rubber tube of sufficient length to allow of free movement of the instrument over the surface of the tube rack. The funnel is conveniently mounted in a ring stand placed in back of the tube rack. Adjustment of the syringe is now made by setting the sheath so that the base of the plunger is stopped on withdrawal at the graduation on the barrel of the syringe corresponding to the amount desired for delivery. It is of great importance to have the entire system completely filled with liquid before any measurements are made or the instrument is employed for filling tubes. This is accomplished for the tube leading from the separatory funnel by either raising and lowering the tube to the level of the contained liquid or stripping it toward the funnel from its tightly compressed end before its attachment to the pipette tip. It is sometimes more difficult to fill the tip and the syringe, especially when using the small sizes, 1 and 2 c.c. syringes, mostly employed in the Wassermann work, on account of the short stroke and consequent



Fig. 3.

slight suction action. This may be facilitated by relieving the valve in the receiving arm by the insertion of small stiff wire; further by unlatching the cap, drawing the plunger completely out and, if necessary, filling the barrel with the solution to be used, a replacement of plunger will thus cause the valves to be released easily and produce a good working condition. Several charges should then be delivered with the tip pointing upward in order to remove the last possible air bubble. Final adjustment is now made by delivering a recorded number of charges into a volumetric flask. If the pipette is to be used for a delivery of one cubic centimeter, one hundred charges should exactly fill a 100 c.c. volumetric flask to its graduation. If the amount falls below this mark, the sheath should be further adjusted by releasing the set-nut and turning the sheath out a few threads. A retrial with the volumetric flask is then performed and repeated until the unit delivery is proved accurate.

Certain precautions are necessary in the use of the pipette. It can be readily perceived that any leakage of the valves would render the delivery

amount inconstant. It is, therefore, important first to have an adjustment of the valves which will insure rapid and perfect action. This is accomplished primarily by the springs on the two valves possessing the characteristics necessary to bring about the proper action under the different conditions in which they function. The spring of the receiving-arm valve is a long spiral of fine spring brass which has an easy resiliance suitable for acting in a total fluid medium; that of the delivery-arm valve is short heavy wire of phosphor bronze which has a strong quick action, although giving easily under liquid pressure, which is suitable for easy release on delivery and for rapid recovery to prevent the intake of air through which medium this valve must also act. It is further advisable to take steps for preventing any solid particles entering the system, which could cause leakage by unseating of the valves. A small disk of 200 mesh copper screen in the base of the separatory funnel as shown in Fig. 3 will do much to prevent this, especially in the use of blood suspensions and serum preparations in which small particles of fibrin are so common. Leakage may be determined by lessened resistance and back-flow in the receiving arm and the presence of air bubbles in the syringe from the delivery arm. Possible leakage may be further checked by multiple recorded delivery into a graduated cylinder; this should always be done where leakage is suspected during the use of the instrument. Correction of the condition is easily accomplished by uncasing the faulty valve (Fig. 1) and freeing it and its seat of the offending matter.

It should be further noted in the use of the pipette that, where different solutions are to be employed, the system should be cleansed with normal salt solution and after filling the stock vessel and tube with the new solution, at least seven charges should be discarded. This will entirely replace the salt solution in the syringe and tip with the new solution.

After use, the system should be cleansed with distilled water and the syringe and tip, either emptied and thoroughly dried or, a more practical method, the syringe and tip allowed to remain filled with distilled water. This will lessen oxidation of the metal parts.

The accuracy and speed of the Automatic Precision Pipette render it valuable in bulk work. Tests have proved the delivery to be constant to 0.0001 gms. This is, we believe, more accurate than is possible by the most careful hand pipetting under similar conditions. It was found that an expert worker with the hand pipette was capable of placing 1 c.c. charges from a 10 c.c. pipette into 100 tubes in an average time of slightly over five minutes. Even an inexpert worker with this instrument can charge 100 tubes in less than one minute. Moreover, the shaking of the tubes necessary in the Wassermann-Bordet reaction after the addition of the blood suspension, occupying about one-half hour to four hundred tubes by hand, is accomplished by the force of the discharge of this reagent from the instrument, thus eliminating this time entirely. In other words, with greater accuracy, one is able to place in the Wassermann tubes, the complement, amboceptor and sheep cells and shake the mixture in a large series of reactions in approximately one-tenth the time necessary for hand pipetting and shaking.



In the Pennsylvania Department of Health Laboratories the instrument has been used mostly in Wassermann work, for complement, amboceptor and sheep cell suspension. It has performed satisfactorily during the past year in over thirty thousand tests. Preparation having been made by assistants, it has allowed this number to be performed by one worker.

The construction and method of operation of the Automatic Precision Pipette suggest other uses to which it may be, with very slight modification, applied, e.g., accurate measurement for dilution, quantitative bottling, accurate animal inoculation, as for diphtheria toxin and antitoxin testing and for blood transfusion.

The author has lately devised a modification of this instrument, shown in Fig. 4, which possesses several points of practical advantage. The syringe

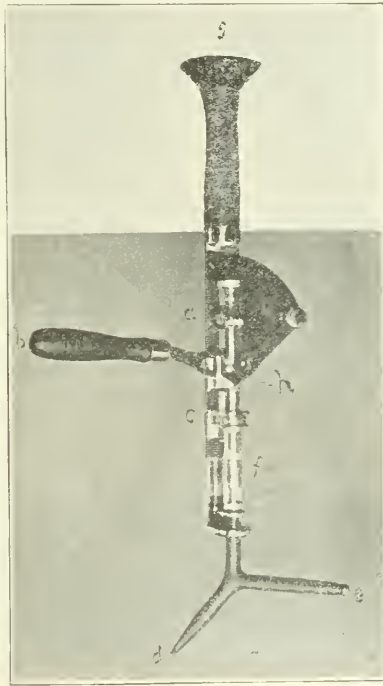


Fig. 4.

(f) is mounted upon a stand carrying the tip (d-e) and supplied with a driving lever (b). The adjustment is made by the set screw (a) which is held firm by a pinch thread and regulates the play of the piston by stopping the rider (h) attached to the lever and piston staff. The syringe is held firmly to the tip by pressure on the cap by an adjustable finger clamp. This arrangement allows the use of any Record Syringe without modification other than removal of the button and screwing the end of the piston staff into the rider, and eliminates breakage to a large extent due to the holding pressure being exerted upon the entire body of the syringe and the jarring of the piston stop being direct, having no straining effect on the syringe as in the former construction.

The operation is also much more smooth and rapid and naturally less tiring to the hand of the worker. The extension of the frame by the body guide (*g*) renders the direction of the pipette into the tubes accurate and steady during operation. This is held against the chest or shoulder during use, the tip is held by the left hand while the right operates the lever.

In running a series of tests whether by hand or with either of the instruments it is wise to fill each test tube and its control successively. With the instrument last described this is most easily done, on account of the direction being governed by the easy bending of the body forward or backward by filling the tubes back and forth over the whole rack.

#### MULTIPLE CENTRIFUGE TUBE HOLDER

The necessity for electrical centrifugalization of specimens of blood to separate the serum from the clot for Wassermann testing is influenced to a considerable extent by how the blood is taken and the size of the collecting tube. Although physicians are directed to take the blood by simple puncture of the finger into tubes 10 mm. in calibre supplied by this department for the purpose, in which case centrifugalization is often unnecessary, many specimens are received at the laboratories not taken this way and requiring centrifugalization. This formerly was done by placing eight specimens at a time in the ordinary centrifuge, which consumed considerable time when handling hundreds of specimens. A multiple tube holder was, therefore, devised, as shown in Fig. 5, to hold twelve specimen tubes instead of one. The holder is so constructed that it is perfectly adjustable to the head of the International Instrument Company Centrifuge. The brass compartment lattice is removable, under which is placed a rubber buffer. The balancing of the holders is simple but care should be exercised to place, insofar as is possible, tubes of approximately equal weight in corresponding compartments of two counterbalanced holders. We have found for nice adjustment of balance the use of small glass beads very convenient.

The saving of time by this device may be easily computed when by this means one is able to prepare ninety-six specimens to the load instead of eight. One might suspect that the exposed area of these holders would increase the resistance and thereby lessen the speed or increase the work of the motor. This is, however, not the case in using the shielded instrument, in which the air content of the shield revolves with the head eliminating this factor to a great extent.

#### A PIPETTE DRYER

The pipette dryer (Fig. 6) consists of a copper box mounted on a wall bracket at a 30° angle. It is twenty inches long, which will accommodate pipettes of the capacities in common use, ten inches wide and five inches deep. The cover is hinged at the top and when open, due to the dimensions and angle will rest securely against the wall in a straight line with the face of the box. The lower posterior angle of the dryer is supplied with small openings for drainage into the sink above which the apparatus should be mounted.

The base carries a screen tray of 200 mesh copper wire to allow of free drainage of the pipettes and prevent breakage of the tips.

The apparatus has eliminated the unsightly practice of keeping used pipettes in glass jars and similar receptacles in the Laboratory, increased the rapidity of drying and lessened breakage. Where desired it may be constructed so that the copper box is removable from the bracket and may be inserted into a sterilizer for sterilization of the contained pipettes.

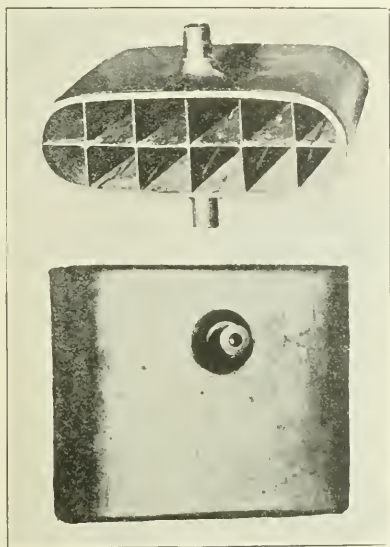


Fig. 5.

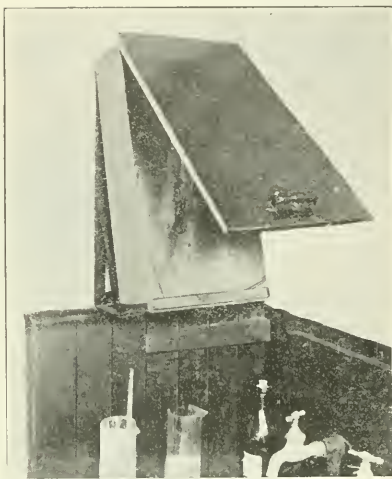


Fig. 6.



Fig. 7.

#### MAILING CASE OPENER

The instrument shown in Fig. 7 deserves passing mention because of its usefulness. It consists of two wood blocks loose-hinged to a base block at the outer ends, which may be fastened to the face of the table or other convenient place. The hinged blocks carry at their inner end a steel-toothed clamp, each representing slightly less than the semicircle of the radius of the cover of the mailing case. They are held away from the base block at the inner end by a light band spring which thereby opens the clamp to a larger diameter than the mailing case cover. To open the mailing case, the cover is inserted between the jaws of the clamp and pushed firmly forward when the hinged blocks coming together as they approach the base will clamp the lid

tightly. A quick turn of the body of the case will then easily unscrew the lid.

Considerable time, trouble and injury to workers' fingers and dispositions have been curtailed thereby.

#### FORMALDEHYDE STERILIZER FOR MAILING CASES

It had been the practice in this Laboratory, as in some others, to discard mailing cases after use for carrying infectious material, viz., tuberculous sputum, etc. This is done to protect the carriers and handlers of these cases in subsequent use against possibility of infection from contaminated cases. Means for sterilizing mailing cases, especially of paper or pasteboard, have been unsatisfactory. Boiling, autoclaving and sterilizing by dry heat are, of course impossible without practically destroying the case, and it has been thought that formaldehyde gas was not penetrating enough to be effective.

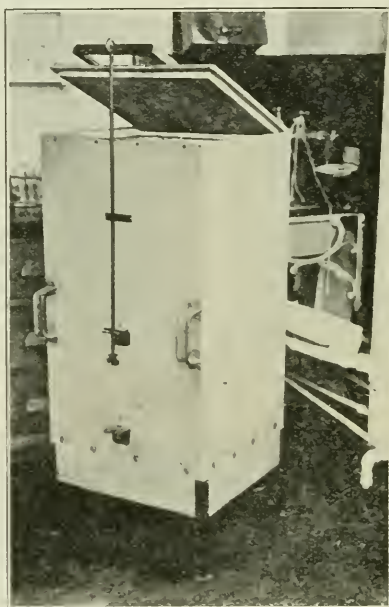


Fig. 8.

The formaldehyde sterilizer shown in Fig. 8 consists of a galvanized iron box 18"  $\times$  18"  $\times$  36" mounted upon iron legs to raise it a convenient distance from the floor. A quarter inch mesh galvanized iron screen tray is inserted on brackets, which hold it about two inches above the bottom of the receptacle which is of copper. The lid, hinged at the back, carries an outer structure to which is attached an iron rod threaded and carrying a thumb nut at its lower end. This may be clamped tightly under the half staples on the face of the box, closing the lid firmly into an inset rim, which is supplied with an asbestos gasket, thereby hermetically sealing the receptacle. The used mailing cases are cast into the apparatus as the specimens are removed from them. One ounce of formalin is then poured down the side of the sterilizer held in a slightly slanting position, so that it reaches the



copper base under the tray. The lid is clamped down and a small Bunsen flame placed beneath for five minutes. The sterilizer is allowed to remain closed overnight when the cases will have been thoroughly sterilized and ready for further use.

Bacteriologic and biologic tests have proved the efficacy of the sterilization. Dried tuberculous sputum thus treated has been proved innocuous. Wet specimens are found to have absorbed an amount of formaldehyde which if inoculated into a guinea pig within twenty-four hours after their removal from the sterilizer is capable of killing the pig in a short time. Specimens allowed to become free from absorbed formaldehyde by longer standing have proved noninfectious.

The marked penetration of the formaldehyde is due to the pressure caused by the application of heat to the hermetically sealed box.

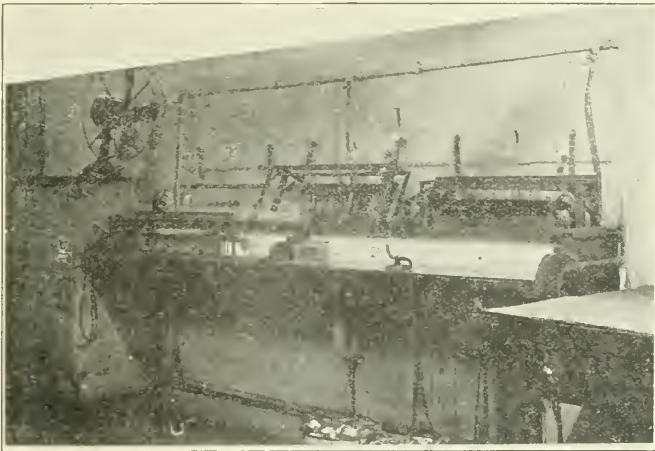


Fig. 9.

Where mailing cases would be otherwise destroyed, the saving of expense by this instrument is easily calculable.

#### STAINING TRAY FOR SMEAR PREPARATIONS

This apparatus was especially constructed for staining sputum smears for tubercle bacilli. When a stain need not be heated the stain-bath method much in use for bulk work is permissible, but where heating is necessary, as for tubercle bacilli, this method is of doubtful value. In the first place, heating the stain to steaming, as is required, must of necessity cause evaporation and deleterious chemical changes in the stain which render it less efficient in subsequent use. Secondly, the heating of a stain to steaming and dipping into it the cold fixed smears produces a physical effect upon the tubercle bacilli in its affinity to take up and hold the fuchsin stain against the action of acids, entirely different from those produced by placing the cold stain upon the smear and heating the glass slide to a temperature sufficient to cause the stain to come to steaming. This difference and the lesser efficacy of the bath method have been well proved.

The apparatus shown in Fig. 9 consists of three separate trays, (as shown at *c*) so constructed as to hold across an open frame twenty microscopic slides. These are held in place, posteriorly by a right-angled edge perforated for drainage and, anteriorly, by a slightly raised beading in the copper frame. The trays, after receiving the smeared slides are placed upon the brackets above the drain basin. The brackets are hinged so that the trays may be tilted sufficiently for thorough drainage during washing and are supplied with a set screw (*A*) to adjust the level in order to hold the stain properly upon the slides. They are raised above the basin a sufficient distance to allow of the application of heat beneath the slides by means of a Bunsen burner of the type shown (*D*). Above the trays are taps (*B*) from which lead rubber hoses for convenient washing. I, II, and III show the trays, in place for staining, tilted for washing and the bracket with the tray (*c*) removed, respectively. Drying of the smears is hastened by means of the fan *F* and the formaldehyde sterilizer for receiving the mailing cases is shown at *E*. In front of the drain basin is a small platform for holding the specimen bottles. These, after use, are placed, uncorked into buckets containing carbolic acid (5 per cent). The back wall of the drain basin is extended well up beyond the taps to protect the surrounding wall completely and collect all drainage.

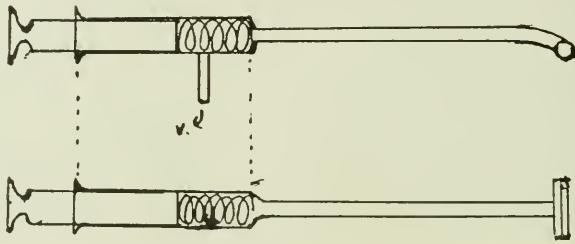


Fig. 10.

This arrangement has proved convenient, rapid of operation and satisfactory. It allows of staining sixty smears in a series under the advantageous conditions obtaining in the handling of a single specimen.

#### VACUUM VACCINE COLLECTOR

Although little used in this laboratory because the production of stock vaccines has not been undertaken by the Pennsylvania Department of Health, the instrument shown in Fig. 10 is of value in laboratories dealing with these products in bulk. It consists of a hollow copper staff bent at the distal end at a 30° angle and carrying a cross tube having a slit  $\frac{1}{32}$ " upon its lower surface. This portion of the instrument is accredited to Lieut. Cole, of the Army Medical School. The novel feature of the instrument represented lies in the mechanical cut-off for the vacuum. This is accomplished by the insertion of the staff into a 10 c.e. syringe of the Ditmer-Robinson type having a solid plunger. A side arm tube is inserted into the barrel of the syringe one and one-half inches from the tip and a brass spring placed inside the syringe beneath the plunger. The spring is constructed to have a contraction which

will allow the plunger to be pressed in beyond the opening of the side arm and a recoil which will force the plunger out beyond this point. Thus, if pressure is applied to the plunger, the vacuum which is attached to the side arm is cut off, and opened upon the release of the plunger. During the lifting of the growth from the culture flasks into suspension, the vacuum is, therefore, cut off by pressure on the piston and release of this allows the vacuum to become active and draw the suspension of bacteria into the vacuum flask.

This instrument may be satisfactorily substituted for the simple staff and rubber tube attachment much in use, where the cut off of the vacuum must be accomplished by hand pressure on the rubber tubing, which is very tiring. It is important that the spring be of strong spring brass wire and that the instrument be cleaned occasionally to insure a free working condition.

#### SHAKING MACHINE

The last instrument to be described in this paper is a shaking machine of a new type which has proved efficient.

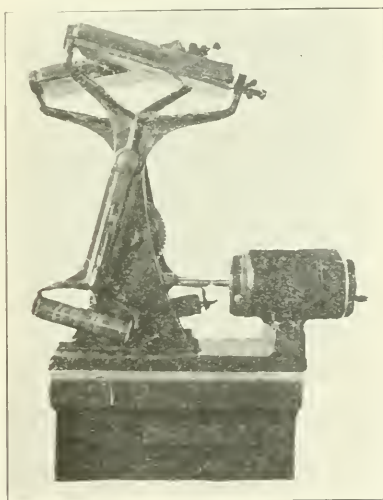


Fig. 11.

The instrument shown in Fig. 11 is compact and based upon mechanical principles which are practical and bring about a method of shaking which closely simulates that produced by the human hand. The arms, carrying the bottle or tube holders at each end, are pivoted somewhat above the middle to a stationary frame and are fenestrated to accommodate the slide of an eccentric attached to a central axle holding the drive gear or wheel. The eccentrics of the two arms are arranged at opposite poles so that the movement of the two sides is compensatory and eliminates almost entirely any vibration of the instrument. The drive gear receives its power direct from the motor by means of a worm. The gears are so proportioned that a motor of 1800 R.P.M. causes an oscillation of the arms at the rate of 200 per minute.

The upper tube holders shown in the figure are for multiple tubes, as in vaccine and sputum work, while the lower are of the type for a single larger bottle. The upper are interchangeable for a still larger single container for greater bulk material, as for antigen extraction. The holders are held in place by fixed points in the rear and by adjustable points anteriorly, held firmly in place by set nuts. The multiple holder is shown more in detail in Fig. 12 with the buffers of sponge rubber (*b*), rubber sheaths and a vaccine bottle (*a*) in place. A 25 c.c. homeopathic vial has proved satisfactory for this purpose. It rests upon a buffer of sponge rubber at the base and the rubber stopper acts as a buffer at the top. The bottles are further retained by covering the entire holder with the lid which is clipped into a slit in the frame posteriorly and held anteriorly by a spring-catch clamp. The holder is divided into eight sections by a brass lattice, allowing of the shaking of sixteen

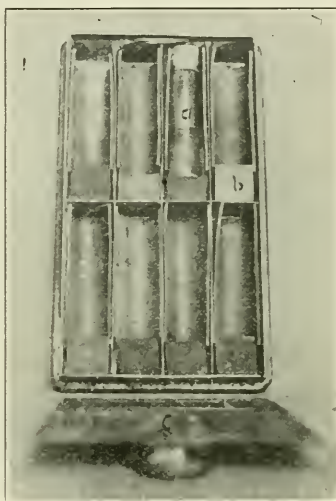


Fig. 12.

specimens to the load. This can be further increased by surmounting the holder with others of similar construction in the place of the lid. The single containers are similarly arranged as to the method of holding the specimen bottle.

The current set up in the shaken fluid or suspension by the machine having a motion through a portion of an arc is in every direction and is consequently efficient. Moreover, the arms being pivoted above the eccentric, move more rapidly when the eccentric slide is at the upper pole than at the lower giving a distinct acceleration at every cycle which tends further toward thorough mixing. - Vaccines are broken up into a homogeneous suspension in a very few minutes. Organic tissue in alcohol, in the presence of glass beads is pulverized almost as completely as if ground in a mortar.

A later model of the shaking machine substitutes a simple pulley for the gear drive and with the motor turned sidewise a belt drive. This lessens the compactness of the instrument to only a slight extent and is more satisfactory for continuous use on account of the lessened friction.



## SUMMARY

Eight new instruments and their uses are described for the facilitation of laboratory procedures, including:

1. An Automatic Precision Pipette.
2. A Multiple Centrifuge Tube Holder.
3. A Pipette Dryer.
4. A Mailing Case Opener.
5. A Formaldehyde Sterilizer for Mailing Cases.
6. A Staining Tray for Smear Preparations.
7. A Vacuum Vaccine Collector.
8. A Shaking Machine.

All of the pieces of apparatus have novel features and their usefulness has been proved in their employment over a period of two and a half years in the Pennsylvania Department of Health Laboratories.

The author wishes to express his indebtedness to Dr. John R. Conover, Assistant Chief of the Pennsylvania Department of Health Laboratories, for his assistance in the devising of the Automatic Precision Pipette; to P. Randolph Hall, Instructor in Mechanical Engineering, and Miles E. Nelson, Mechanician in the Electrical Engineering Department, University of Pennsylvania, for the mechanical construction in the experimental work; and to George H. Wahmann, of Baltimore, for the construction of the instruments.

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## SOURCES OF ERROR IN THE EPSTEIN METHOD FOR BLOOD SUGAR DETERMINATION AND A MODIFIED TECHNIC

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By C. M. WILHELMJ, ST. LOUIS, Mo.†

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THE Epstein method<sup>1</sup> for the determination of blood-sugar, using .2 c.c. of blood, is in reality a modification of the original Lewis and Benedict procedure, and its simplicity makes it an ideal method for use in clinical investigation. Since it is often desirable to obtain blood at very short intervals during sugar tolerance tests, with the least possible inconvenience to the patient, this is the method of choice if it proves to be sufficiently accurate. In a few preliminary determinations however, the results obtained seemed too high, and it was considered advisable to check the method before further work was done.

Briefly the technic described by Epstein uses .2 c.c. of blood, obtained from the lobe of the ear or the finger, which is put into sodium fluoride solution and laked by adding water up to the 1 c.c. mark on the tube. The proteins are then precipitated with picric acid which is added to a total volume of 2.5 c.c. After filtration 1 c.c. of the filtrate is carefully heated over the naked flame. *The contents of the tube are boiled until all but 2 or 3 drops of the solution is evaporated. One-half cubic centimeter of 10 per cent sodium*

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\*Received for publication, October 13, 1921.

†From the Medical Service of the Jewish Hospital.

carbonate solution is then added and the tube heated again until the contents are concentrated to a small volume equal to about 2 or 3 drops. The color changes from yellow to deep red or reddish brown and the reaction is complete. With careful rinsing the contents of the tube are then transferred to the graduated tube of the hemoglobinometer and compared with the standard solutions.

In checking the method, accurate standard glucose solutions were prepared which contained respectively 50, 80, 100, 200, 250, and 280 mg. of glucose in 100 c.c. of water. Two-tenths of a c.c. of each solution was then treated according to the Epstein technic. In every case the percentage obtained was found to be considerably higher than expected. Each step in the technic was carefully checked, and in order to eliminate the personal equation, the color comparisons were usually made with a second party to check the result; but even with the most careful manipulation the results continued high, and a characteristic set of readings is given in Table I.<sup>1</sup> The source of error was naturally first sought in the apparatus, and therefore the pipettes, graduated tubes, and standard solutions were all carefully checked but were found to be sufficiently accurate. *Furthermore the error was not constant when duplicate determinations were made on the same solution.*

It seemed evident that the source of error must be sought in the method. The step most likely to give this error is the one where 1 c.c. of the picrated filtrate is concentrated by boiling to 2 or 3 drops, and  $\frac{1}{2}$  c.c. of 10 per cent sodium carbonate added, and the solution again boiled down to a volume of 2 or 3 drops. When the amount of alkali used here ( $\frac{1}{2}$  c.c. of 10 per cent sodium carbonate) per unit volume of blood (.08 c.c.), is compared with the amount used in the original Lewis and Benedict method<sup>2</sup> we find that they use the equivalent of  $\frac{1}{4}$  c.c. of 10 per cent sodium carbonate per .08 c.c. of blood, while the Myers and Bailey modification<sup>3</sup> uses a trifle more than  $\frac{1}{4}$  c.c. of 10 per cent sodium carbonate per .08 c.c. of blood. In the Epstein technic at least twice the amount of alkali needed, per unit volume of blood, is used, and in addition the filtrate representing this amount of blood is concentrated by boiling to about  $\frac{1}{5}$  its original volume both before and after the addition of alkali.

The monosaccharides are unstable in alkaline solutions and the degree of instability is directly proportional to the amount of alkali and the tem-

TABLE I  
RESULTS ON GLUCOSE SOLUTIONS

PER CENT OF GLUCOSE IN SOLUTION	PER CENT BY EPSTEIN TECHNIC	PER CENT BY MODIFIED TECHNIC
.05	(1) .061 (2) .079	(2) .055
.08	(1) .084 (2) .108	(2) .082
.10	(1) .114 (2) .126	(2) .104
.20	(1) .210 (2) .222	(2) .202
.250	(1) .260 (2) .272	(2) .258
.280	(2) .310	(2) .286

perature. If a simple solution of glucose is heated with an alkali, decomposition and condensation occur, producing caramel and other substances which give a yellow or brownish color to the solution. These dissociation products, before condensation takes place, have strong reducing ability, and if some reducible substance (picric acid) is present, they will reduce it as they are formed. Small amounts of glucose, as dilute as .05 per cent, when boiled with  $\frac{1}{2}$  c.c. of 10 per cent sodium carbonate, will give a yellow or brown color, showing that this amount of alkali is sufficient to produce the decomposition. Without a doubt the combination of too much alkali plus the greater heat obtained over the naked flame, as used by Epstein, causes some of the glucose to be broken into these several particles, each of which may bring about the reduction of a molecule of picric acid, and thus slightly more reducing material is produced from a given amount of glucose.

With the above source of error in mind, Epstein's technic was modified as follows:

Using the Epstein saccharimeter, 2 c.c. of blood, obtained from the lobe of the ear or the finger, is drawn into the pipette. The blood is then transferred to the graduated mixing tube which contains a small amount of powdered potassium oxalate. The blood pipette is rinsed 2 or 3 times with water which is added to the blood in the tube. Distilled water is added up to the 1 c.c. mark and the blood is laked. Saturated picric acid is then added to the 2.5 c.c. mark and the tube thoroughly shaken. The contents are then filtered. One c.c. of the protein free filtrate is transferred to the boiling tube and concentrated over the naked flame, *before the addition of sodium carbonate*, to a volume of  $\frac{1}{2}$  c.c., cooled under running water, and  $\frac{1}{4}$  c.c. of 10 per cent sodium carbonate added. The tube is then put in a water-bath, in water at room temperature, and left in for *fifteen minutes or more\** from the time at which vigorous boiling begins. At the end of this time it is removed, cooled under running water, and the contents transferred to the graduated tube. The boiling tube is then rinsed with 2 or 3 drops of distilled water at a time and these rinsings transferred to the graduated tube of the hemoglobinometer, by means of a small pipette with a capillary tip. These rinsings are repeated with 1 or 2 drops of water until the volume in the graduated tube has been made up to the 50 mark (1 c.c.) The solution is then compared with standard tubes which accompany the saccharimeter and the per cent of glucose calculated according to directions sent with the apparatus.

We have substituted a smaller boiling tube than the one supplied with the Epstein apparatus and have graduated it at  $\frac{1}{2}$  c.c., so that the concentration of the filtrate can be accurately done. By using the smaller tube and transferring the small rinsings with a capillary tipped pipette, we find that the  $\frac{1}{4}$  c.c. is sufficient to wash all the material from the boiling tube. Table I<sup>2</sup> shows the comparative results obtained by the original Epstein technic and this modification, when used on standard glucose solutions.

The effect of creatinine on sugar determinations by the Lewis and Benedict method has been the subject of much discussion, and the results using the three methods, i.e., Epstein, Modified Epstein, and the Benedict modification of the Lewis and Benedict, are given in Table II. The amount of creatinine added to each solution (10 mgs.) if it all reacted to produce picramic acid, should add one point in the second figure to the per cent of

\*It was noticed on several occasions that boiling for less than fifteen minutes did not complete the reaction.

glucose. From these results we see that the Epstein method probably causes reaction of all the creatinine plus the error referred to above, and that the modification reacts to creatinine in full amounts. However when we consider that creatinine is normally present in amounts from 1 to 2 mgs., we see that even a high normal amount would add only .002 to the per cent of glucose.

In Table III the figures obtained on blood are shown. The results obtained by the Epstein technic are seen to be both high and inconstant. *This variability is apparently due directly to the amount of concentration after addition of the alkali, for in some of the duplicate determinations the concentration was not carried as far as 2 or 3 drops and here slightly lower figures were obtained.*

TABLE II  
RESULTS OBTAINED USING GLUCOSE AND CREATININE

SOLUTION CONTAINING PER 100 C.C.	PER CENT BY EPSTEIN TECHNIC	PER CENT BY MODIFIED TECHNIC	PER CENT BY BENEDICT MODIFICATION OF LEWIS AND BENEDICT METHOD
50 milligrams glucose	.085	.064	.056
+ 10 milligrams creatinine			
80 milligrams glucose	.129	.092	.089
+ 10 milligrams creatinine			
100 milligrams glucose	.140	.106	.095
+ 10 milligrams creatinine	.128	.106	.105
200 milligrams glucose	.240	.208	.204
+ 10 milligrams creatinine			
250 milligrams glucose	.285	.258	.252
+ 10 milligrams creatinine			

In most of the cases duplicate determinations by the modified technic are given, using the average of readings with the .05 and .1 per cent standards, and are seen to check very well.

In order to be sure that the modified technic was reacting with all of the sugar present in blood, known amounts of glucose were added to blood in which the sugar had previously been determined, and the percentage obtained compared with the calculated value; the results are shown in Table IV. It is seen that the modification recovers the added glucose, and that even the greatest differences do not account for the discrepancy between it and the original Epstein. A comparison with the figures of Höst and Hatlehol<sup>4</sup> show that the results are as good as can be expected with a colorimetric method.

In 1916 Epstein<sup>5, 6</sup> published two articles in which the blood sugar was studied in patients before and after operation. The results published in the first paper were obtained by the Lewis and Benedict method and in the second by the Epstein procedure. It is seen that his results, on patients before operation, show the same difference between the two methods that we have demonstrated, an average of .093 per cent by the Lewis and Benedict and .126 per cent by the Epstein technic. But for some unaccountable reason this discrepancy has been passed by without comment or criticism.



TABLE III\*  
RESULTS ON BLOOD

CASE	EPSTEIN TECHNIC PER CENT	MODIFIED TECHNIC PER CENT
1	.148	.106
2	.140	.082
3	.148 (.1 standard)	.114 (.1 standard)
Acute Articular Rheumatism		.105 (.05 standard)
		.109 average
	.140 (.1 standard)	.116 (.1 standard)
		.105 (.05 standard)
		.110 average
4	.146 (.1 standard)	.10 (.1 standard)
Healthy		.103 (.05 standard)
		.101 average
	.140 (.1 standard)	.10 (.1 standard)
		.105 (.05 standard)
5	.118 (.1 standard)	.102 average
Healthy		.10 (.1 standard)
		.099 (.05 standard)
		.099 average
		.10 (.1 standard)
6	.146 (.1 standard)	.10 (.05 standard)
Healthy		.106 (.1 standard)
		.104 (.05 standard)
		.105 average
	.136 (.1 standard)	.104 (.1 standard)
		.109 (.05 standard)
7	.148 (.1 standard)	.106 average
Hypopituitary		.114 (.1 standard)
		.116 (.05 standard)
		.115 average
8	.152 (.1 standard)	.130 (.1 standard)
Typhoid Mastoiditis Pyelitis		.134 (.05 standard)
		.132 average
	.170 (.1 standard)	.134 (.1 standard)
		.134 (.05 standard)
		.134 average
9	.138 (.1 standard)	.096 (.05 standard)
Healthy		
	.166 (.1 standard)	.102 (.1 standard)
10	.196 (.1 standard)	.102 (.05 standard)
Infection of Foot		.166 (.1 standard)
	.196 (.1 standard)	.168 (.1 standard)
		.167 average
11	.180 (.1 standard)	.158 (.1 standard)
Hernia 9th Postoperative Day		.160 (.1 standard)
		.159 average

\*None of the cases are fasting specimens, in all the blood was taken shortly after breakfast or dinner.

## CONCLUSIONS

The Epstein method for blood-sugar determination when checked against standard glucose solutions and blood, gives results which are always too high and the per cent of error is not constant. This source of error is due (1) to the use of too great a concentration of alkali (2) too much heat (3) concentration of the protein free filtrate to too small a volume both before and *after* the addition of alkali.

The modification given brings the accuracy of the Epstein method reasonably up to that of the Lewis and Benedict procedure.

TABLE IV  
RESULTS WHEN KNOWN AMOUNTS OF GLUCOSE ARE ADDED TO BLOOD

CASE	BLOOD AS DRAWN PER CENT	GLUCOSE ADDED	PER CENT CALCULATED	PER CENT BY MODIFIED TECHNIC	DIFFERENCE
7	.115	.1 c.c. of .040 per cent sol. added to 1 c.c. of filtrate	.165	.166	+ .001.
6	.105	.1 c.c. of .040 per cent sol. added to 1 c.c. of filtrate	.155	.170	+ .015
	.105	.3 c.c. of .040 per cent sol. added to 1 c.c. of filtrate	.255	.260	+ .005
12	.071	.1 c.c. of .040 per cent sol. added to 1 c.c. of filtrate	.121	.130	+ .009.
		.2 c.c. added	.171	.166	- .005
		.3 c.c. added	.221	.210	- .011
		.4 c.c. added	.271	.258	- .013
		.8 c.c. of .020 per cent sol. added to blood in laking (.064 mg. added to 1 c.c. of filtrate)	.151	.150	- .001
8	.132	.1 c.c. of .040 per cent sol. added to 1 c.c. of filtrate	.182	.186	+ .004.
9	.102	.1 c.c. of .040 per cent sol. added to 1 c.c. of filtrate	.152	.160	+ .008
		.3 c.c. added	.252	.250	- .002.
10	.167	.2 c.c. of .040 per cent sol. added to 1 c.c. of filtrate	.267	.274	+ .007.
11	.159	.2 c.c. of .040 per cent sol. added to 1 c.c. of filtrate	.259	.264	+ .005.

It should be emphasized that work which has been done in the past on the sugar tolerance in various diseases, using the Epstein method, has probably given results which are too high and inconstant.

In conclusion I wish to thank Dr. Jerome E. Cook for the opportunity and facilities which he offered for the conduction of this investigation, and for his valuable suggestions and kindly interest.

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## THE BLOOD-SUGAR CONTENT OF CAPILLARY BLOOD AS COMPARED WITH THAT OF VENOUS BLOOD\*

BY ISAAC NEUWIRTH, B.S., AND ISRAEL S. KLEINER, PH.D.

A NUMBER of methods have, in recent years, been devised for the determination of sugar in very small amounts of blood.<sup>1</sup> These methods have enabled the clinician to make blood-sugar tests without entering a vein and have been widely used. Quite recently one of the present authors (K) has described another micromethod<sup>2</sup> for blood-sugar which simplifies and shortens the procedure to a considerable extent.

Naturally the blood for a micro-determination is taken from the finger or ear-lobe. This is, of course, capillary blood mixed with a small amount of tissue fluid. Although there is no good reason to suppose that this will differ materially from venous blood in sugar content, it seemed worth while to test the point, in order that we may know whether it is correct to use venous and capillary blood-sugar values interchangeably.

Bang<sup>3</sup> has summarized the older literature on comparisons of arterial and venous blood sugar, which might be expected to throw more light on the subject since capillary blood should not differ markedly from arterial. In general the arterial figures are higher than venous but the variations between different investigations in which different technic and different animals were employed is tremendous. In fact, Bang says,<sup>4</sup> "Die Frage über den relativen Gehalt des Muskelarterien und Venblutes in der Ruhe ist also noch nicht experimentell gelöst und bedarf neuer Untersuchungen." There seems to be no record of comparisons between capillary and venous blood-sugar, however.

The present experiments were performed upon twenty medical students, during the course of their usual work at college. All except subjects 7 and 8 were males. Capillary blood was collected from a finger and about one minute later venous blood was drawn from a vein of the same arm.<sup>†</sup>

That we were dealing with arterial (capillary) and with venous blood, was evident from the color of the samples. Analysis of the blood was begun at once; the Kleiner micromethod, a modification of Benedict's picrate-picric acid technic, was used. The results are given in Table I.

It will be seen that of the 20 comparative tests, five show identical values, in two the venous figure exceeds the capillary and in thirteen the capillary figure is higher. The greatest difference is 0.02 per cent, found in two instances. In other words in eighteen out of the twenty experiments the results did not differ over 0.01 per cent, and in the other two only 0.02 per cent. Evidently

\*(From the Department of Physiological Chemistry of the New York Homeopathic Medical College and Flower Hospital).

†We wish to thank Dr. Leo M. Powell and Mr. Joseph I. Nevins for obtaining the blood samples and also the students who acted as subjects.

Received for publication, Aug. 18, 1921.

the capillary blood-sugar closely parallels the venous and for all clinical purposes can be accepted as equivalent.

An average of all twenty capillary figures is 0.136 per cent as against 0.130 per cent for the venous. This result, as well as the fact that in the

TABLE I

COMPARISON OF BLOOD-SUGAR CONTENT OF CAPILLARY AND VENOUS BLOOD OBTAINED SIMULTANEOUSLY, USING MICROMETHOD THROUGHOUT

NO.	CAPILLARY BLOOD	VENOUS BLOOD	TIME ELAPSED SINCE LAST MEAL
1	0.14%	0.13%	2 hours after breakfast
2	0.10	0.09	5 hours after breakfast
3	0.12	0.11	4 1/4 hours after breakfast
4	0.12	0.11	3 hours after breakfast
5	0.12	0.12	1/2 hour after eating 1 apple and 4 3/4 hours after breakfast
6	0.12	0.12	1/2 hour after lunch
7	0.12	0.12	no breakfast; candy during morning
8	0.11	0.11	5 hours after breakfast
9	0.13	0.12	3 hours after breakfast
10	0.15	0.14	3/4 hours after lunch
11	0.17	0.16	1/2 hour after lunch
12	0.16	0.14	1 hour after lunch
13	0.12	0.13	4 hours after breakfast
14	0.12	0.12	4 hours after breakfast
15	0.12	0.13	4 hours after breakfast
16	0.13	0.12	3 3/4 hours after breakfast
17	0.14	0.13	3/4 hours after lunch
18	0.18	0.16	1 hour after lunch
19	0.17	0.16	3/4 hour after lunch
20	0.18	0.17	1 hour after lunch

TABLE II

COMPARISON OF MICRO AND MACRO BLOOD-SUGAR METHODS

NO.	MICROMETHOD (KLEINER)	MACROMETHOD (MYERS-BAILEY)
1	0.13%	0.13%
2	0.09	0.09
3	0.11	0.10
4	0.11	0.10
5	0.12	0.12
6	0.12	0.12
7	0.12	0.13
8	0.11	0.12
9	0.12	0.13
10	0.14 (0.135)	0.11 (0.114)
11	0.16	0.17
12	0.14	0.13
13	0.13	0.12
14	0.12	0.11
15	0.21	0.20
16	0.11	0.11
17	0.14	0.13
18	0.11	0.12
19	0.12	0.11
20	0.13	0.12
21	0.16	0.15
22	0.27	0.29 <sup>a</sup>
23	0.10	0.11 <sup>a</sup>
24	0.10	0.10
25	0.50	0.48 <sup>a</sup> ; 0.49
26	0.21	0.20



majority of the experiments, the capillary blood was slightly higher, harmonizes with the theoretic expectation. Indeed, Bang has calculated that arterial blood should contain on an average 0.01 per cent more than venous. Our averages closely approximate his calculation.

We wish also to present some figures showing the degree of accuracy to be obtained with the method employed. In this series (Table II) blood was analyzed by both the micromethod mentioned above, and by the Myers-Bailey modification<sup>5</sup> of the Lewis-Benedict method. In the former 0.2 c.c. and in the latter 2.0 c.c. of blood are used. It will be seen that the results agree closely enough for clinical purposes.

#### SUMMARY

In twenty individuals the capillary blood-sugar was found to closely parallel the venous blood-sugar. For clinical purposes the two values may be considered identical.

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# *The Journal of Laboratory and Clinical Medicine*

VOL. VII.

MAY, 1922

No. 8

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Washington, D. C.

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## EDITORIALS

### *Newer Conceptions Concerning Shock*

**S**TUDIES, facilitated by the abundance of clinical material during the World War, have developed new conceptions of the causation of shock and have stimulated research work on this subject. The more important recent work and the newer theories have been summarized and discussed recently in these columns. The weight of evidence indicates that traumatic shock follows liberation into the circulation of a hypothetical substance derived from damaged tissues. This substance resembles histamine both in its action and its chemical constitution. One important action apparently is the production of a general capillary dilation. The diminished blood volume found in shock appears to be due first to stagnation of the circulating blood in the increased capillary beds, and second to loss of fluid from the blood through hyperpermeable endothelium, damaged by the toxic substance. Additional work within the last few months has served to substantiate these findings and has facilitated further understanding of the underlying processes.

There are still those who maintain that the lowered blood pressure characteristically found in shock is related to abnormal functioning of the adrenal

glands, as adrenal exhaustion. Rich has recently shown quite conclusively that in standardized experimental shock in which all variables are kept as nearly constant as possible, adrenalectomized animals reacted to shock exactly as did normal controls, the time required for the production of shock and the character of the blood pressure curves being the same in both types of animals. The experiments were so timed that they were not complicated by the subsequent hypotension characteristic of adrenal insufficiency. He concluded from his rather extensive experiments that disordered adrenal function is not a factor in the production of shock. In shock it is not epinephrin insufficiency which accounts for the fall in blood pressure.

Rich concluded that cardiac failure is not a factor in the production of shock. The heart functioned satisfactorily at all times, and in every experiment in which the animal was allowed to die in shock, the immediate cause of death was respiratory rather than cardiac. Stimulation of the central end of one cut vagus nerve caused reflex cardiac inhibition even in the most extreme degrees of shock. This vagus reflex was elicited even when the systolic pressure was as low as 20 mm. Section of the vagi was found to consistently increase the heart rate even in profound shock. Division of the vagi before the traumatism causing shock did not alter in any way the characteristic blood pressure curve. Rich also concludes that exhaustion of the vasomotor center is not a primary factor in shock. Stimulation of the sciatic nerve still produced a definite pressor response when the blood pressure was as low as 22 mm.

Granting that low blood pressure is a characteristic of shock and that in all probability it is a result of diminished circulating blood volume, what are the effects of this reduced blood pressure and volume on the various organs? Following diminution of blood volume either from hemorrhage or from shock there is at the outset no change in the blood pressure. It is only after an appreciable interval that the pressure commences to fall. For a time the normal tension is maintained by constriction of the peripheral vessels. This diminishes the capacity of the blood system. As the total blood volume falls below the minimum capacity of the system there may be a final relaxation of vascular tone from the diminished activity of the vasomotor system. This would serve to depress still further the falling blood pressure.

The vasoconstriction which, following moderate diminution in blood volume, serves to maintain the normal tension is not necessarily evenly distributed throughout the organs of the body. Thus, Gesspell has shown that with the removal of ten per cent of the estimated blood volume from an animal the reduction of volume flow per minute through the submaxillary glands amounts to sixty per cent of the basal flow, while there is little or no simultaneous lessening of the volume flow through the brain or the heart. In the presence of normal arterial pressure the blood vessels of the central nervous system and of the heart do not contract. Thus, extensive contraction in other tissues of the body serves to maintain an adequate blood supply to these organs.

Camon and Cattell point out that the three essential elements which the blood conveys to the body cells are food, water and oxygen. The cells are

able to sustain a prolonged absence of fresh food supply in the blood without disturbance of function. To a lesser extent this is also true of fresh water supply in the blood. Oxygen on the contrary is essential and must be supplied at all times. The supply of oxygen in the capillaries becomes diminished as the blood supply lessens, first, because of primary vasoconstriction in the peripheral vessels, and second, as the symptoms progress, from a diminished blood pressure and a resultant diminished rate of blood flow. Cannon and Cattell raise the important question as to whether in shock there is actually an insufficient supply of oxygen to the tissues.

Diminution of the head of pressure in the circulatory system will diminish the rapidity of flow and will thereby increase the length of time required for the transportation of oxygen from the lungs to the tissues. The accumulation of blood in the capillary system results in prolonged contact between the erythrocytes and the tissues. Theoretically, therefore, more oxygen will at first be extracted from the blood by the tissues and less will be delivered thereafter per unit time.

It is a fact first demonstrated by Henderson in 1910 that in shock there develops a reduction of alkali reserve in the blood, paralleling the diminution in blood pressure. It has been shown by various observers that at the same time there occurs an increase of lactic acid in the blood. There is considerable evidence to show that an insufficient oxygen supply to the tissues results in the formation of lactic acid. The lactic acid normally formed from muscular contraction cannot be burned to carbon dioxide and water if insufficient oxygen be present. Cannon and Cattell suggest that the increase of lactic acid resulting from diminished oxygen supply may account for the reduced alkali reserve.

They have found experimentally that a reduction of blood alkali does not occur until after the blood pressure has fallen to below a critical level of approximately 80 mm. Hg. Below this level the reduction in alkali is comparatively rapid. The same critical level has been found by Aub to exist for fall in basal metabolism in shock. The inference is that with a systolic blood pressure above 80 the blood volume flow is sufficient to supply oxygen to the tissues, but that below this point diminished oxygen delivery results in the production of lactic acid and a resultant acidosis. If hemorrhage exists in addition to shock the critical level of blood pressure is somewhat higher, around 90 mm. Morphine for some unexplained reason lowers the critical level and delays the onset of reduction in alkali reserve.

With a blood pressure below the critical level the various tissues of the body are receiving an insufficient oxygen supply. If this condition is continued over a prolonged period permanent damage will ensue. The length of time during which body cells may be deprived of oxygen and yet later return to normal varies for different tissues. One of the most sensitive cells of the body is the pyramidal cell of the cerebral cortex. Eight minutes of anemia kills many of these cells. The cells of the medulla are more resistant. From eight to thirteen minutes of anemia produces only slight changes or none at all, but after twenty or thirty minutes alterations occur which prevent com-



plete recovery. The ganglion cells of the sympathetic nervous system may be completely deprived of blood for as long as an hour and yet return to normal function with the return of normal blood supply. Of course in shock the oxygen want is not absolute.

The damaging effects of brief anoxemia on nerve cells has been shown by morphologic study. Cannon and Cattell have demonstrated physiologic deterioration of these cells following prolonged periods of hypotension below the critical level. They stress the gradually damaging effects of persistent hypotension and the prime importance of early treatment of low blood pressure in shock.

Henderson and Haggard have emphasized the importance of deficient oxygen supply in the symptoms from severe hemorrhage. The fall in blood pressure may parallel that of shock uncomplicated by hemorrhage. The blood pressure may be returned approximately to normal by intravenous infusion of various fluids such as normal salt solution, sodium bicarbonate and gum acacia solution, but none of these substances is as efficacious in overcoming the effect of hemorrhage as is the infusion of blood itself. Gum acacia is not without distinct dangers. Henderson and Haggard believe that the important element administered is the red blood cells themselves which through their oxygen carrying capacity relieve the oxygen want of the blood. They also find a reduction in the alkali reserve of the blood coincident with the reduction in oxygen carrying capacity, but their explanation differs radically from that offered by Cannon and Cattell. Oxygen deficiency in the circulating blood induces a condition of air-hunger so that more oxygen may be supplied to the blood through the lungs. This increased ventilation washes out a relatively large amount of the carbon dioxide, leaving an excess of alkali in the blood. The resulting alkalosis is compensated for by the disappearance of the excess alkali from the blood into the tissues or into the urine. They suggest that oxygen administration would decrease the ventilation of the lungs thereby conserving carbon dioxide and that both the carbon dioxide and alkali content of the blood would then increase, with resulting recovery. The most successful way to increase the oxygen in the blood would be by increasing the oxygen carrying elements, by transfusion.

The newer work on shock and hemorrhage both by members of the "acidotic" school and by those of the "acapneic" school agree definitely on the importance of a sufficient oxygen supply to the tissues in the prevention of permanent damage and a fatal outcome.

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—W. T. V.

### *Medical Fellowships*

SINCE the war the laboratories in medical schools and those in charge of them have been unable to secure the services of satisfactory assistants. The fact that so many clinical teachers in our medical schools have gone on full time and are paid much larger salaries than the laboratory men, has greatly discouraged the latter, because they have been unable to secure or retain desirable assistants. If this condition should continue it would mean a great dearth of laboratory teachers and of laboratory investigations in our medical schools and indeed we are beginning to feel this already.

In view of these facts, the Rockefeller Foundation and the General Education Board have placed in the hands of the National Research Council the sum of \$100,000 annually to be used for medical fellowships. These fellowships will be open to citizens of both sexes of the United States and Canada. It will be expected that applicants will possess either the degree of Doctor of Medicine or Doctor of Philosophy, or a grade of scholarship corresponding to one of these degrees. The fellowships will not be given to universities or institutions, but to individuals, and the size of the stipend will be determined in each individual case. The fellowships are to be administered by a board, the chairman of which is to be, ex officio, the chairman of the Division of Medical Sciences, of the National Research Council. The amount of each stipend will be determined by the scholarly promise of the individual, his past work and his family obligations. It is intended that each stipend shall be large enough to enable its holder to live in comfort while doing his fellowship work. Inasmuch as the chief purpose of this endowment is to supply future teachers in medical schools, fellows will be required to devote part of their time to teaching, but it is understood in every case that research work is essential. Best teachers are those who have had some creative experience.

The sum appropriated will permit of the appointment of some thirty or more medical fellows each year and it is to be hoped that during the five years for which provision has been made, the scheme will prove so successful that it will be continued. The success of this project will depend upon its administration. The board will need to exercise good judgment in the selection of fellows. Ten or fifteen years from now the establishment of these fellowships should begin to markedly tell upon the character and the productive scholarship of the faculties of our medical schools.

The fellow will have the privilege of selecting the institution in which, and the man or men under whom, he will do his work. The university in which the work is done will be expected to furnish facilities. The Administrative Board of the National Research Council will determine whether the selection of the fellow is wise and we have no doubt that the Board will extend to him advice along these lines.

Anyone desirous of securing further information concerning these fellowships should write to the Chairman of the Division of Medical Sciences, National Research Council, 1701 Massachusetts Avenue, Washington, D. C.

—V. C. V.

### *A Research Fellowship in Bacteriology*

THE Society of American Bacteriologists, at its December, 1921, meeting in Philadelphia, appropriated a fund for the support of a Research Fellowship in pure bacteriology. While excellent work is being carried on in many places, most of the problems under investigation have as their aim a practical application and there are, therefore, many gaps in our knowledge of fundamental principles.

The Society of American Bacteriologists, believing it to be the duty of its members to fill these lacunae, provides that the line of work carried on under its fund must concern a purely scientific and fundamental phase of bacteriology, although a certain latitude of choice will be permitted, conditioned by the previous training and the desire of the research fellow himself.

Applicants for the fellowship must have the degree of B.S. or its equivalent. The successful candidate, through arrangements now being made, will receive academic credits for the work done in carrying out his research, from a university of recognized standing. One Hundred Dollars per month will be available for the living expenses of the fellow.

The Washington Branch of the Society of American Bacteriologists has taken over the cultures which for the past ten years have been maintained at the American Museum of Natural History by Professor Winslow, and has deposited this collection at the Army Medical Museum, where facilities have been arranged for its housing and maintenance. The following have consented to act as Curators of this collection:

Major G. R. Callender, Curator of the Army Medical Museum;

Dr. George W. McCoy, Director Hygienic Laboratory, U. S. Public Health Service;

Major H. J. Nichols, Army Medical School;

Dr. J. M. Sherman, Dairy Division, Bureau of Animal Industry.

These and other members of the Society in and near Washington will do volunteer work and the research fellow will do part time work in maintaining the collection. No charge will be made for the cultures. In making requests, the classification of the Society should be followed as far as possible. Inquiries about cultures should be addressed to the Department of Bacteriology, Army Medical Museum, 7th and B Streets, S. W., Washington, D. C.

Applications for this fellowship will be passed upon by the following board:

Dr. Victor C. Vaughan, Chairman Division of Medical Sciences, National Research Council, Chairman;

Captain C. S. Butler, Medical Corps, U. S. Navy, Commandant, Naval Medical School;

Dr. George W. McCoy, Director Hygienic Laboratory, U. S. Public Health Service;

Dr. J. R. Mohler, Chief of Bureau of Animal Industry;

L. A. Rogers, (President Society of American Bacteriologists) in charge of Research Laboratory, Dairy Div., Bureau of Animal Industry;

Colonel Joseph F. Siler, Medical Corps, U. S. Army, Division of Sanitation, Office of Surgeon General of the Army;

Dr. Erwin F. Smith, Pathologist in Charge Laboratory of Plant Pathology, Bureau of Plant Pathology.

Applications for this fellowship should be sent to the chairman of the above-named committee.

—V. C. V.

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### *Where Do the Vitamins Come From?*

IT is still a question in what plants and animals the vitamins originate. Is there any animal which can synthesize a vitamin? If this can be answered in the negative, we may ask, what plants synthesize vitamins, and what are the conditions essential to such a synthesis? As early as 1913 it was suggested by Funk that vitamins secreted in cow's milk do not originate in the body of the animal, but come solely from the cow's food. If this be true, it follows necessarily that the richness or the poverty of cow's milk in vitamins depends solely upon the food supplied the animal. Since Funk made the suggestion, numerous investigators have experimentally studied the subject and, so far as we know, have quite unanimously come to the conclusion that mammals at least, do not synthesize vitamins. In 1916 McCollum and his colleagues concluded that vitamins A and B pass into the milk only as they are present in the food of the mother. Two or three years later Steenbock and colleagues ascertained that the amount of fat-soluble vitamin A present in butter is subject to seasonal variation and especially to the food supplied the animal. In 1920 Hess and Unger advanced the idea that an insufficiency of vitamin A in milk might be due to the winter food of the cow, and about the same time Barnes and Dutcher and their coworkers reported a marked seasonal variation in the antiscorbutic properties of cow's milk; indeed, it seems to be quite uniformly shown that the vitamins in cow's milk at least, are determined by the food supplied the cow. Recently, Kennedy and Dutcher<sup>1</sup> have used for their rat-feeding experiments two types of milk; one produced on a ration typical of that used on some farms during the winter season and known to be deficient in its vitamin content, and a second representing that produced on a ration carrying ample amounts of vitamins A and B. These investigators state their conclusions as follows: "(1) The presence of vitamins A and B in cow's milk is entirely dependent upon their occurrence in the ration. (2) Stall fed cows will produce a milk rich in vitamins, provided their ration consists of a proper combination of grains and leafy foods. (3) A vitamin-rich milk is not necessarily correlated with access to pasturage. (4) Ten c.c. per day of either winter or summer milk is adequate to furnish either vitamin A or B to a rat, provided the ration of the cow carries each in amounts adequate to meet her requirements. (5) Five c.c. of the same milk that pro-



duced normal growth when used on a higher level does not furnish enough of either vitamin A or B to meet the requirements of growing rats. (6) The effect of the vitamin is not necessarily one of appetite stimulation but rather stimulation of metabolic processes which promote growth."

In other words, Kennedy and Dutcher have shown that the difference between summer and winter milk, so far as vitamin content is concerned, is due to the food supplied the cow, and winter milk may be quite as rich in vitamin as summer milk, provided the cow is supplied with proper food during the winter.

Admitting that mammals do not synthesize vitamins, it has been held by some that low forms of plant life, and especially the yeasts, are concerned in this process; indeed, the taking of yeast to supply vitamins has become a fad among the semiintelligent or semi-well informed people of this country. Yeast in various forms is now a staple article of diet among those who think they are keeping up with science. The question is, does yeast synthesize vitamins? If so, what are the particles used by the yeast in this synthesis? Some years ago, Eijkman, after he had shown that rice polishings contain large amounts of antineuritic vitamin, grew yeasts on these polishings and found, much to his surprise, that the polishings lost their antineuritic properties; in other words, the yeast had simply extracted from the rice polishings the vitamins. The yeast had not made any vitamins.

Recently, Eijkman and certain coworkers<sup>2</sup> have gone into this subject, and report: "It seems, therefore, that yeast not only takes eventually its antineuritic factor as such from the culture medium but that it is not even capable of synthesizing the vitamin unless the medium contains at least the products of decomposition of the vitamin by heating."

This means that even the yeast plant does not possess the property of creating the ultimate constituents which are essential in the synthesis of vitamins. If this finding be corroborated, it must follow that yeasts vary in their vitamin content according to the food supplied them and that in order to get vitamins it would be safer to use the plant substances which we know to contain them.

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—V. C. V.

### *Excess Protein and Mammary Secretion*

HARTWELL,<sup>1</sup> on evidence which is not altogether free from criticism, holds that lactating animals should not have too much protein in their food. By too much protein she seems to imply that more than thirty-three per cent of the dry food is protein, and thinks that on such a food the nursing young are likely to die. Her experiments were made on rats and the excess of protein was provided for by the addition to the food of edestin, blood and

<sup>1</sup>Biochemical Jour., 1921, v, 565.

egg albumen, gelatin, and home-made gluten. Such dietaries apparently rendered the milk poisonous and finally checked its flow. The toxicity of the mother's milk is demonstrated by the development of spasms in the young. The cessation of the flow of milk is shown by the loss in weight of the young and the emptiness of their alimentary tracts after death. The mother is likely to put on weight at the very time the young are developing spasms and dying. Much has been said and written about an excess of protein in our food and physiologists have been divided on this point, some claiming that ordinarily we eat too much protein.

Chittenden has been the most forceful advocate of a low protein diet. So far as we know, the experience of all armies in the World War fails to show that any harm came to any one from an excess of protein in his food, while it is shown that much harm came to many on account of too little protein in the food. We are told that too little protein in the food is the cause, or at least is a factor in the causation, of pellagra, and possibly of other deficiency diseases. Now comes Hartwell and tells us that too much protein in the food is bad during lactation. It does no harm to the mother, but it kills the young. We are not convinced by Hartwell's evidence. She herself seems somewhat in doubt about it. Having used commercially prepared proteins in order to make the food excessive in this constituent, she admits that these commercial products may have been denatured in some way, and that this may account for her results. She is also conscious of the fact that her dietaries were deficient in fat and in vitamins, and possibly the harm to the babies might have come from this cause rather than from an excess of protein in their food. At least, this is a matter which needs further investigation. So long as proteins in the food are split up into amino acids in the small intestine, none of which are poisonous and all of which in excess are rapidly eliminated, we must, for the present at least, decline to accept Hartwell's conclusion.

—V. C. V.

# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, JUNE, 1922

No. 9

## ORIGINAL ARTICLES

### FOCAL EMBOLIC GLOMERULONEPHRITIS\*

BY MARGARET WARWICK, M.D., ST. PAUL, MINN.

DURING the last two decades medical investigators have given renewed attention to the subject of nephritis, particularly in respect to its etiology and classification. However, one very important type of this disease, viz., focal embolic glomerulonephritis, has received but little attention. This neglect is probably due to the fact that these cases are relatively rare, are not always carefully studied and even then are not recognized as a distinct pathologic entity but are classed as examples of diffuse glomerulonephritis.

Focal embolic glomerulonephritis was first described by Löhlein<sup>1</sup> in 1910, when he reported eight cases and discussed the condition in detail. It was again mentioned briefly by Aschoff<sup>2</sup> who described this special type of kidney lesion associated with "ulcerative" endocarditis. Volhard and Fahr<sup>3</sup> included the subject in their monograph, accompanying their description by microphotographs. But it was not until the classical treatise of Baehr<sup>4</sup> appeared in 1912, with a study of 34 cases, that this type of kidney lesion was accorded a secure and definite place.

The lesion, which is usually prominent and easily recognized by the trained, careful observer, consists of an involvement of some loops of some of the glomerular tufts while the remaining loops and glomeruli remain entirely normal. A rather characteristic feature is that various stages of the lesion appear in neighboring glomeruli or even adjoining loops of the same glomerulus. Therefore it presents a sharp contrast to the usual glomerulonephritis where practically all the glomeruli present throughout the tuft the same stage of the same lesion.

The first change in a glomerular loop is a swelling of the endothelial

\*Read before the American Association of Pathologists and Bacteriologists, Cleveland, March 26, 1921.

Received for publication, August 22, 1921.

cells which gradually lose their cell boundaries and show either pyknosis or disappearance of their nuclei and a final fusion into a granular and later a hyaline mass. Special stains will frequently demonstrate in this stage a small amount of fibrin. Then occurs almost simultaneously a proliferation of the neighboring cells, particularly of the adjoining part of Bowman's capsule, which finally becomes attached to the affected loop. This sclerosed and fused loop and adjoining capsular surface frequently gives to the glomerulus the "half-moon" appearance mentioned by German observers. Frequently one area of the tuft may be entirely sclerosed while another loop shows the early degeneration of the endothelial cells and yet another is unaffected. Löhlein feels that the process may spread from one loop to another but Baehr maintains that each loop must be separately affected either at the same or at different times. Entirely sclerosed glomeruli are often seen, representing a finished or healed process.

The condition of the tubules and interstitial tissue depends upon the extent of the glomerular involvement. If a glomerulus is almost or entirely sclerosed, the corresponding tubules will undergo atrophy and connective tissue replacement with some lymphocytic infiltration. Other tubules will show marked dilatation. One very constant feature in this condition, however, is the persistent presence in the tubules of red blood cells which have been traced by Baehr to the affected glomerulus. The number of glomeruli affected varies from 2 per cent to 75 per cent. In a few cases search through several blocks of tissue is necessary to demonstrate the lesions.

The theories of etiology all center about the presence of the *Streptococcus viridans* in the circulating blood. Since this organism presents a dry tenacious mass of bacteria upon growth, Baehr feels that the lesions are produced by the small masses of bacteria occluding the lumen of a glomerular loop. The proof of this theory lies in the staining of the bacteria *in situ* in the glomerulus, a procedure not easily accomplished. Löhlein found bacteria in one afferent vessel of a glomerulus but was unable to show them in the tuft itself. In Baehr's large series no bacteria were found in tissues with formalin fixation but in 5 specimens fixed in alcohol bacteria were demonstrated. In my series all efforts in that direction were unsuccessful.

The offending streptococcus in these cases is usually the one about whose accurate classification there is much confusion and discussion, i.e., the *Streptococcus viridans* or "green-producing (on blood agar) streptococcus"; also known as the *Streptococcus mitis*, or "endocarditis coccus," so frequently associated with a chronic or subacute endocarditis. Löhlein demonstrated it in 3 of his 8 cases while the other 5 yielded a streptococcus which was not classified upon blood media. Baehr found it in 25 of his 34 cases in either the blood stream, vegetations upon the heart valve or both. Two other cases of "chronic" or "subacute endocarditis" yielded a pure blood culture of *B. influenza* and the *Gonococcus* respectively. The kidneys failed to show characteristic lesions. Also none of these lesions were found in the kidneys in 54 cases of acute endocarditis, about half of which were proved by blood cultures or by cultural or microscopical examinations of the vegetations to be of bacterial origin.



The lesion, other than that described in the kidney, usually associated with this bacteremia is an endocarditis. Löhlein, Aschoff, and Volhard and Fahr described an ulcerative endocarditis with partial destruction of the mitral and frequently the aortic valve leaflets but Baehr finds what he following Libman designates as a "subacute bacterial endocarditis." Such a condition is described by Libman,<sup>5</sup> and is so named in preference to the older terms "chronic," "ulcerative," or "malignant" or "infectious" endocarditis. He finds in this condition the mitral valve usually involved with a tendency for the vegetations to spread up on the left posterior wall of the auricle and chordæ tendineæ rather than the valve leaflet itself. The aortic valve is also frequently affected but here the lesions are more variable and with more tendency to ulceration than the mitral. The vegetations are of a yellowish, greenish, pinkish or reddish color, vary in size and, as they grow older, are firmer and more grayish.

Libman also considers that these cardiac lesions may proceed to a healing or to a "bacteria-free" stage, particularly if for some reason the bacterial stage is very short, due to low virulence of the organism or high resistance of the host. In such cases the kidneys present the typical lesions but they are all in the healed stage. However, in spite of the fact that the vegetations and kidney lesions are healed, the case usually shows no clinical improvement and goes on to a fatal termination.

The only other lesions mentioned as being associated with subacute bacterial endocarditis and focal embolic glomerulonephritis are constant enlargement of the spleen and frequent infarction of the spleen, kidneys and brain. And the only constant clinical finding outside of the symptoms of endocarditis is the presence of persistent microscopic blood in the urine in addition to albumin and casts.

In the autopsy records of the Department of Pathology at the University of Minnesota during the past 5 years, 10 cases of focal embolic glomerulonephritis were found during the routine examination of microscopic sections of all organs. Doubtless prolonged and detailed search might have revealed others in which fewer glomeruli were involved. But it is of interest to note that 10 attracted attention in a routine examination and therefore are sufficiently typical to warrant study.

In our series there were 9 males and 1 female. The ages varied from 18 to 61 years, with an average of 38 years. In all 10 cases the heart was enlarged from 365 grams (in a small female) to 860 grams with an average of 515 grams. The mitral valve was involved alone 5 times; the mitral and aortic together 4 times, while the aortic alone was involved but once. The lesion consisted of vegetations which were sometimes accompanied by ulcerations of the valve leaflets. In 5 cases (50 per cent) vegetations and ulcerations occurred together but in the other 5 vegetations occurred alone, thus disproving the older idea that the endocarditis associated with focal embolic glomerulonephritis was usually of the "ulcerative" type. The vegetations varied markedly in appearance and position upon the endocardium. The majority were fresh and friable while some were more firm and showed

beginning calcification in certain areas. In some instances the vegetations were very numerous and extended over the auricular wall as described by Libman. In one case, for example, the auricular wall alone was extensively involved while the valve leaflets remained free. But in other cases the vegetations were small and discrete and situated on the edges of the leaflets. One heart showed only 2 small vegetations on the mitral and one large one on the aortic. In another, yellowish white vegetations associated with calcareous nodules were found on the mitral only.

There was as much variability in the extent and position of the ulcerations which occurred on either valve or both. The ulcerated area was frequently very small and situated either on the edge or in the center of the leaflet. On the other hand in one instance both the mitral and aortic valves were entirely destroyed except for ragged tags attached to the endocardium. Considering the great variability in the appearance of these hearts one is justified in the assumption that there is no one definite cardiac lesion, or type of heart, associated with focal embolic glomerulonephritis, but rather that any of these types of endocarditis may be associated with the specific form of bacterium that causes the kidney lesions. As a further substantiation of such a conclusion, Fahr<sup>6</sup> in a recent paper describes in a case of septicemia and meningitis (streptococcic and meningococcic) typical glomerular lesions but *no* endocarditis.

As these autopsies were performed by various members of the staff, both past and present, in various places and upon bodies often embalmed, often partly decomposed, there is necessarily a lack of uniformity in the bacteriologic studies. In only 5 of the 10 were blood cultures taken either just before or just after death and in each of these 5 a streptococcus was obtained. In 4 of the 5 it proved to be the *Streptococcus viridans* but in one of these there was also present the *Streptococcus pyogenes*. In the fifth case, however, one showing the classical ulceration and vegetations of the mitral valves and presenting most typical lesions, *Streptococcus hemolyticus* was obtained from the blood stream, showing that streptococci other than the *viridans* may be responsible for focal embolic glomerulonephritis.

All observers apparently agree that a streptococcus is most commonly associated with these kidney lesions but in many instances the type was not determined. Löhlein, although usually finding a streptococcus, expresses the opinion that other organisms might be responsible for the lesions. Baehr, however, found the *Streptococcus viridans* or "endocarditis coccus" in 25 of 34 cases (66 per cent). On the other hand, two similar cases of subacute bacterial endocarditis, yielding from the blood stream *B. influenza* and a *Gonococcus* respectively, failed to show the kidney lesions. The one case in our series proves rather conclusively that the "hemolytic" as well as the "viridans" type of streptococcus may produce similar changes in the kidney, just as it is becoming more generally recognized that changes in other organs are not always diagnostic of any special type of invading parasite.

Many infections in other parts of the body show very dissimilar lesions caused by the same organism affected by the ever different ratio existing

between the virulence of the bacterium and the resistance of the host. Theoretically, it is possible that these lesions, showing largely a degeneration and only slight signs of exudative inflammation, may be caused by any organism of low grade virulence irrespective of type. Because the *Streptococcus viridans* is usually of low grade virulence it is perhaps the most common offender, but other organisms, as the *hemolyticus*, may, under auspicious conditions as to virulence and resistance, produce the same lesions. The fact that Baehr and Libman failed to find typical kidney lesions with endocarditis due to other organisms does not disprove the fact that these same organisms may, if properly attenuated, occasionally resemble the *Streptococcus viridans* in their effect on the kidneys.

In all the cases of this series where a history was available, the urine showed albumin, hyaline and granular casts, occasional leucocytes and persistent red blood cells, but in no case was gross blood present—a point of differentiation from cases of acute hemorrhagic nephritis. In 5 of the 10 cases a functional (phenolsulphonephthalein) test was done and it varied from a trace to 39 per cent (trace, 5 per cent, 25 per cent, 34 per cent and 39 per cent, respectively). When one considers the wide variability of the lesions and that very many or only a few of the glomeruli may be partly or completely destroyed, it is easy to understand that not only will the function vary widely in different cases but that occasionally it may closely approximate the normal.

In all 10 cases the spleen was enlarged, varying from 220 to 650 grams, with an average of 416 grams. This splenic enlargement has also been noted by other observers. Chronic passive congestion does not wholly account for the splenic enlargement which, together with the persistent, otherwise unexplained presence of red blood cells in the urine, may prove to be points of value in making a clinical diagnosis. Emphasis should be laid on the importance of careful bacteriologic studies in these as well as other types of infectious lesions. Blood cultures during life with the finding of *Streptococcus viridans* may prove of distinct diagnostic significance while, at the postmortem examination, careful cultures of blood, valvular thrombi and even kidney may give valuable information.

In our series there were also 5 cases showing multiple infarcts of the spleen, kidneys and often brain. This confirms the already mentioned theory that these lesions are the result of simple tiny emboli lodging in the loops of the glomeruli. The proliferation of cells and fibrinous exudate indicate an inflammatory process dependent upon the presence of bacteria. Fahr suggests that the process may be first a simple infarction with secondary proliferation as the result of the death of tissue, bacteria not necessarily being present. Our most rational conclusion would be that these tiny emboli, whether they contain living or dead bacteria, produce lesions which are largely obstructive in effect, although in practically every case some toxic element, either present in the embolus or formed by bacteria multiplying after it lodges, must be assumed to account for the evident inflammatory phenomena. At any rate, bacterial proliferation is not a prominent feature, as special staining methods often fail completely to demonstrate their presence.

Because of the tendency of the "endocarditis coccus" to form rather dry tenacious clumps and because he has succeeded in staining them *in situ*, Baehr feels that the embolus consists entirely of bacteria. However, it is more reasonable to believe, as does Fahr, that some emboli are largely aseptic while others may be largely composed of bacteria. In his case, already mentioned, where there was no heart lesion but an overwhelming infection with streptococci and meningococci, there might be masses of bacteria plugging up the glomerular tufts while the resistance of the tissue was too low to produce the ordinary reaction of abscess formation or of acute glomerulonephritis. The many stages encountered frequently in the same kidney lesions or even the same glomerular lesions are accounted for by the fact that these tiny emboli are continually fed into the blood and may continue to produce new lesions. Occasionally the vegetations upon the heart valves may heal or become calcified, thus preventing the production of further emboli and consequently preventing the formation of new lesions. The result in the kidney is the final presence of healed lesions only, a condition observed by Baehr and Libman.

In a very recent publication Baehr has noted in a study of 77 cases of focal embolic glomerulonephritis that 9 were complicated by an acute glomerulonephritis and 2 by a chronic diffuse nephritis, evidently superimposed upon the embolic type. He suggests that the embolic process may lower the resistance of the kidney, particularly the glomeruli, making them easy prey to the subsequent acute infection. At the same time in such cases the symptoms of endocarditis were obscured by anasarca, edema, etc.

This study, as well as those which have been quoted, shows that this subject is as yet unfinished and further investigations are needed to solve all the questions involved. In this as well as many other as yet unsolved problems in the science of medicine the hope of a satisfactory explanation lies not only in the valuable fund of knowledge obtained over the autopsy table but in its correlation with the clinical findings and closer cooperation between pathologist and clinician.

#### SUMMARY

1. Focal embolic glomerulonephritis is a definite clinical and pathologic entity.

2. It is usually associated with an endocarditis which, however, has no characteristic appearance and it is even possible to have the kidney lesion without any endocarditis being present.

3. It is usually caused by a *Streptococcus viridans* but may be produced as well by the *Streptococcus hemolyticus*.

4. The lesions in the kidney probably depend for their character upon a bacterium of low grade virulence rather than upon any definite type of organism. The *Streptococcus viridans*, being naturally of low grade virulence, is the most common offender.

5. It is possible that lesions may be formed as the result of a mechanical



closure or infarction of the glomerular loop and not be dependent upon the presence of living bacteria.

6. The only positive clinical findings are the enlarged spleen and persistence of microscopic blood in the urine, associated with subacute bacterial endocarditis.

7. The phenolsulphonephthalein test results vary widely with the number and part of the glomeruli affected, at times giving only a trace, at others closely approximating normal.

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## THE RÔLE OF HEXAMETHYLENAMINA IN THE PRODUCTION OF HEMATURIA\*

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DURING the epidemic of influenza which occurred at the Naval Academy in the winter of 1920-21, and towards the close of this epidemic, there were admitted to the hospital four cases of hematuria of sudden onset and uncertain etiology. These patients entered the hospital at intervals of a few days and the sudden occurrence of the hematuria and close association of the patients suggested a common etiologic factor.

Upon investigation it was found that they had all been ill for several days before admission to hospital with a mild type of influenza and had all received, in addition to other medication, a solution of *Mistura Glycyrrhizæ Composita* and hexamethylenamina. The average dose of hexamethylenamina in this solution was 10 grains and was administered three times a day in one of the cases and four times a day in three of the cases. A fifth case was also found which was not admitted to the hospital but whose case was investigated and whose history showed that she had been receiving this same solution three times a day. This case, occurring outside the Naval Reservation, and having received the same medication as the four cases admitted to hospital, suggested the medication as the common etiologic factor. A careful examination, together with a close survey of the history in these cases, tended to rule out an infectious agent as the cause of the hematuria, and indicated that the hexamethylenamina which all of these patients had received, was the direct cause. The clinical course of the cases and subsequent findings confirmed this opinion.

It appears during the influenza epidemic that one of the routine medications dispensed to the milder cases of influenza which were not admitted to hospital was a solution of *Glycyrrhizæ Composita* and hexamethylenamina. This solution contained ten grains of hexamethylenamina to the dram and was dispensed, as a rule, in dram doses three or four times a day. There were approximately four hundred cases treated with this solution. The usual period of treatment varied from one to five days and the average was two days. Of this number treated, there developed five frank cases of hematuria, four of which were admitted to hospital.

While it has been known for many years that the administration of hexamethylenamina may be followed in some cases by hematuria, little hesitancy, as a rule, is observed in administering this drug. The reason is probably the

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Received for publication, August 27, 1921.

fact that this untoward effect of hexamethylenamina is so infrequently noted. However, when this condition is produced the discomfort and inconvenience suffered by the patient are quite marked and are certainly worth avoiding if possible.

This drug was introduced in 1895 by Bordet and has rapidly come into universal use as a urinary antiseptic. It is very rapidly absorbed and may be found in the urine very shortly after administration. When the urine is strongly alkaline it sometimes fails to act.

Bastedo, in discussing the untoward effects of this drug, states "In acid urine it sometimes so increases the acidity as to make the urine irritating, or sets free enough formaldehyde to do this; and marked vesical pain, frequent burning micturition, bloody urine and defoliation of the bladder mucous membrane have been reported."

Richardson showed that in the presence of existing nephritis, there was no increase in albumin and casts following the administration of hexamethylenamina.

Coleman, in 1903, reported untoward sequelæ consisting of irritation of the stomach, diarrhea, and abdominal pain; irritation of kidneys and bladder, with hematuria and hemoglobinuria; headache, ringing in ears and a rash like that of measles.

Crow reports that of 95 cases receiving an average dose of 75 grains a day, seven developed painful micturition and hematuria. He also noted in some cases skin lesions, acute catarrh of mucous membranes and gastric irritation.

Frothingham, in 1909, reported that very large doses could be given to guinea pigs without toxicity, although the stomachs were prone to become ulcerated and to bleed. Our experiments with guinea pigs have yielded different results from his reports.

Burnam states that a 50 per cent solution of the drug is not irritant.

Cushny, in discussing the action of hexamethylenamina states, "No symptoms arise from ordinary doses of urotropine, but large quantities have occasionally given rise to pain and discomfort in the bladder, and more rarely to hematuria; the irritant here is not the urotropine itself but the formaldehyde liberated by it."

Brown reports two cases of vesical hematuria from 10 grains three times a day for eight days.

Morton noted burning in the urethra and frequent micturition from 24 grains a day.

Biss, in 311 typhoid cases, noted irritation and slight hematuria twice. The general opinion seems to be that the blood comes from the bladder and not from the kidneys.

The following five cases of hematuria, following the administration of hexamethylenamina, are reported. The time elapsing between beginning the administration of the drug and the appearance of hematuria varied in these cases from one to seven days. The earliest case developed after the second

dose of 10 grains and the latest case developed on the seventh day following the administration of hexamethylenamina in 10 grain doses four times a day.

CASE I.—Age nineteen, white, male, unmarried. *Complaint*.—Frequent, painful urination of bloody urine. *Family History*.—Negative. *Personal History*.—Mumps and measles in childhood. Health has been excellent up to present illness. Patient has always been a resident of Maryland. Visited Panama and Hawaii in the summer of 1920.

*Present Illness*.—Three days prior to admission to hospital patient complained of cough and general malaise and received treatment consisting of a mixture of hexamethylenamina and Mistura Glycyrrhizæ Composita. He received a total of ten grains of hexamethylenamina four times a day. On the fourth day of this treatment he noticed a frequent desire to urinate, the urine appearing reddish in color, and there was a burning sensation more marked at the end of urination. There was no nausea or vomiting and patient felt well in all respects other than the discomfort referable to bladder.

*Physical Examination*.—Upon admission to hospital, temperature, pulse and respiration were normal. Patient was well nourished and muscular; mucous membranes and skin clear; no enlargement of spleen. Abdomen soft and no areas of tenderness. Pupils equal and react to light and accommodation; tongue clean; tonsils and pharynx negative; teeth in good condition; heart and chest negative; bones and joints negative; reflexes both superficial and deep normal; eye grounds negative. Cystoscopic examination showed several hemorrhagic areas at base of bladder, particularly in the region of the left ureter. These areas varied in size from 1/2 to 2 cm. in diameter and were irregularly distributed over the bladder wall, appearing as bright reddish blotches. There was considerable tenesmus and irritation around the neck of the bladder. The vault and lateral surfaces of the bladder appeared normal.

*Laboratory Data*.—Radiograph of chest negative. Sputum constantly negative for tubercle bacilli. Blood examination shows no abnormal findings. Noguchi test negative. The total quantity of urine for twenty-four hours was 1,050 c.c. It showed a heavy trace of albumin and an abundance of red blood cells. No casts were found. The total acidity was 80 and the  $p_H$  5.2. The urine was negative for organisms and parasitic ova.

*Clinical Course*.—Patient was afebrile during entire period in hospital. During the first three days following admission there was almost constant desire to urinate, patient getting up three or four times during the night. There was pain and a burning sensation around the neck of the bladder following urination. The urine passed was bloody but contained no clots. Red cells persisted microscopically in the urine for fourteen days and gradually became less, until on the eighteenth day there were no red cells demonstrable microscopically or chemically.

CASE II.—Age twenty-one, male, unmarried. *Complaint*.—Frequent and painful urination with bloody urine. *Family History*.—Negative. *Personal History*.—Measles and whooping-cough in childhood. Mumps in 1918, no complications. Tonsillitis and influenza in fall of 1918, good recovery. Patient is a resident of South Carolina.

*Present Illness*.—Five days prior to admission to hospital patient had a cold and was confined to bed. During this time he was given a cough mixture consisting of hexamethylenamina and Mistura Glycyrrhizæ Composita three times daily. He received ten grains of hexamethylenamina in this mixture at each dose. On the fifth day after taking this medicine patient noticed some pain and frequency of urination, the urine containing blood and mucus. There was no nausea or vomiting and no symptoms referable to abdomen except the bladder symptoms.

*Physical Examination*.—Upon admission to hospital T. 100, P. 96, R. 20. Patient was well nourished and muscular. Skin and mucous membranes clear; lymph glands not enlarged and spleen not palpable. Abdomen soft and there were no areas of tenderness. Tonsils and posterior pharynx slightly congested; pupils equal and react to light and accommodation; teeth in excellent condition; heart and chest negative; bones and joints negative; reflexes normal; eye grounds negative.



*Laboratory Data.*—Radiograph of chest shows some thickening of primary and secondary bronchi to the bases, characteristic of an old bronchitis; otherwise negative. Sputum constantly negative for tubercle bacilli; blood examination showed a moderate leukopenia. No abnormal cells or parasites found. Noguchi reaction negative. Total quantity of urine in twenty-four hours was 1,100 c.c., showing heavy traces of albumin and an abundance of red blood cells. No casts or parasites present. It was noted that the urine was highly acid, total acidity being 7.8 and the  $p_H$  5.4. Cystoscopic examination shows hemorrhagic areas at base of bladder, varying in diameter from 1/2 to 3 cm. These areas were bright red in color and irregularly distributed over the base of the bladder and in the region of the ureters. The vault and lateral surfaces of the bladder were normal.

*Clinical Course.*—On the day following admission temperature, pulse and respiration were normal. The urine rapidly cleared. The patient noticed on the second day in the hospital that the first urine was clear and only a few drops of bloody mucus followed at the end of urination. Ten days after admission the urine was entirely free from red blood cells, both microscopically and by chemical tests. Daily tests of the urine showed  $p_H$  values rarely exceeding 5.4.

CASE III.—Age twenty-one, white, male, unmarried. *Complaint.*—Frequent and painful urination of bloody urine. *Family History.*—Negative. *Personal History.*—Scarlet fever, measles, mumps and diphtheria in childhood. Influenza in October, 1917, at which time patient was given hexamethylenamina in doses of 10 grains three times a day for ten days, following which he developed a hematuria which lasted three days. Acute arthritis in March, 1920, duration two weeks, good recovery. Patient had not been outside the United States until 1920, when he visited the tropics on the battleship cruise.

*Present Illness.*—One week prior to admission to hospital patient had an acute coryza and received treatment consisting of Mistura Glycyrrhizæ Composita and hexamethylenamina, 10 grains to each dose, which was administered four times a day for a period of seven days. On the morning of the seventh day he complained of pain and a burning sensation on urination, and passed a few drops of blood at the end of urination. There was no abdominal tenderness nor symptoms referable to gastrointestinal tract. Patient complained of slight sore throat and general malaise.

*Physical Examination.*—Upon admission to hospital T. 101, P. 88, R. 20. Patient well nourished and well developed muscularly; skin and mucous membranes normal; spleen not palpable. Abdomen soft and no areas of tenderness or tumor formation. Pupils equal and react to light and accommodation. Eye grounds negative; tongue clean and tonsils show moderate hypertrophy; teeth in excellent condition; heart and lungs negative; bones and joints negative; reflexes normal.

*Laboratory Data.*—Radiograph of chest shows no parenchymal lesion of lungs and no evidence of tubercle. Sputum negative for tubercle bacilli and throat cultures negative for Klebs-Loeffler bacilli. Blood cultures negative and white and differential count showed no abnormality. Stools negative. The urine voided in twenty-four hours was 900 c.c., showing a total acidity of 7.8 and a  $p_H$  of 5.8. It was dark red in color and showed a heavy trace of albumin and an abundance of red blood cells. Urine negative for tubercle bacilli and negative for acetone and diacetic acid. No parasitic ova or casts found. Cystoscopic examination was impossible as patient showed marked spasm and tenderness which precluded the passage of the cystoscope.

*Clinical Course.*—On the day following admission temperature became normal and continued normal until the eleventh day, when patient developed a mild attack of influenza with temperature rising to 102 and returning to normal after four days. On the first few days following admission the urine voided was well mixed with blood and contained a few small clots. There was tenesmus, a burning sensation and marked frequency of urination. On the third day the first urine passed was clear, blood being noticed only at the end of urination with an occasional small clot. The red cells rapidly disappeared, the urine becoming clear and on the fifteenth day only a few erythrocytes were seen microscopically. On the eighteenth day the urine was entirely free from blood microscopically and chemically, and patient was discharged from the hospital on the twenty-fifth day entirely well.

CASE IV.—Age twenty, white, male, unmarried. *Complaint*.—Frequent, painful urination of bloody urine. *Family History*.—Negative. *Personal History*.—Mumps, measles and whooping-cough in childhood. Frequent attacks of tonsillitis over period of several years. Influenza in 1920; good recovery. No venereal history and no previous hematuria. Patient is a resident of Tennessee and has never lived in the tropics but visited Panama, the Philippine Islands and Cuba in 1920.

*Present Illness*.—Five days prior to admission to hospital patient was confined to his room with a mild attack of tonsillitis and during this period received, in addition to local treatment to throat, a medicine consisting of Mistura Glycyrrhizæ Composita and hexamethylenamina four times a day. He received at each dose a total of 10 grains of hexamethylenamina. On the third morning he noticed a frequent desire to urinate and the appearance of a few drops of bright red blood, together with a painful, burning sensation at end of urination. On the fourth day all urine passed was bloody and patient had much pain and discomfort. There were no gastrointestinal symptoms other than anorexia.

*Physical Examination*.—Upon admission to hospital T. 99.2, pulse and respiration normal. Patient was well nourished and muscular; skin and mucous membranes were clear; there was no enlargement of lymph glands or spleen; pupils were equal and reacted to light and accommodation; eye grounds negative; tongue clean; tonsils hypertrophied and showed a slight follicular exudate; teeth in good condition; heart and chest negative; bones and joints negative; reflexes, both superficial and deep, were normal. Cystoscopic examination showed numerous hemorrhagic areas at base of bladder and around both ureters. These areas were bright red in color with irregular outlines varying from  $\frac{1}{2}$  to 2 cm. in diameter. The vault and lateral surface of the bladder appeared normal.

*Laboratory Data*.—Radiograph of chest negative except for some thickening of the bronchi to the bases. Sputum negative for tubercle bacilli. Blood examination showed nothing abnormal; Noguchi test negative; throat cultures negative for Klebs-Loeffler bacilli but showed presence of streptococcus hemolyticus. The total amount of urine in twenty-four hours was 800 c.c. It was dark red in color with a heavy trace of albumin and an abundance of red blood cells. The total acidity was 68 and the  $p_H$  5.5.

*Clinical Course*.—Patient showed evening elevation of temperature for several days following admission, which could be attributed to his tonsillitis. The hematuria continued for three days with considerable pain and tenesmus upon urination, the urine being dark red in color but free from clots. The red cells gradually disappeared and on the twelfth day urine was entirely negative, both microscopically and chemically for blood, and the symptoms referable to the bladder had subsided. Patient was discharged to surgical service for tonsillectomy before returning to duty. Following tonsillectomy he was returned to duty and has shown no subsequent signs or symptoms referable to urinary tract.

CASE V.—Age 51, white, female, married. *Complaint*.—Frequent, painful urination of bloody urine. *Family History*.—Negative. *Personal History*.—Had usual diseases of childhood. No serious illness except pneumonia in 1917, from which she made a good recovery. Has three children, all normal births, no complications. Patient is a resident of Maryland and has never been outside the United States.

*Present Illness*.—While under treatment for acute bronchitis by her family physician she was placed upon a medicine containing Mistura Glycyrrhizæ Composita and hexamethylenamina. Each dose of this mixture contained ten grains of hexamethylenamina, which was given three times a day. Following the second dose of the medicine patient noticed a frequent desire to urinate and severe pain in the region of the bladder. On the following morning, after four doses of the medicine, she noticed the urine was deeply colored with blood and the pain and tenesmus were more severe.

*Physical Examination*.—Temperature, pulse and respiration were normal. Patient was well nourished and robust; skin and mucous membranes were clear; there was no enlargement of the lymph glands or spleen; pupils were equal and reacted to light and accommodation; tongue clean; tonsils and pharynx negative; teeth in good condition; heart negative. Chest showed a few râles in both bases, otherwise negative. Bones and joints negative;

reflexes, both superficial and deep, were normal, eye grounds negative. Cystoscopic examination was not feasible in this case. Blood pressure 156-90.

*Laboratory Data.*—Sputum negative for tubercle bacilli. Blood examination showed no abnormal findings; Noguchi test negative. The total quantity of urine in twenty-four hours was 960 c.c., and showed a heavy trace of albumin and numerous red blood cells. The total acidity was 78 and the  $p_H$  5.4. The urine contained an occasional finely granular cast. No tubercle bacilli or parasitic ova were demonstrable.

*Clinical Course.*—The hematuria, pain and tenesmus gradually subsided, and on the tenth day the urine was entirely negative except for the presence of an occasional granular cast which persisted up to time of discharge.

In reviewing the history of these five cases an attempt was made to ascertain the factors or predisposing causes, if any such existed, which would lead to a hematuria. It is a well-known fact that hexamethylenamina, to be effective as a urinary antiseptic, must have an acid medium in order that formaldehyde may be liberated. The factor of urinary acidity appeared very suggestive, particularly as all of these cases had shown a rather high total acidity as well as a high hydrogen-ion concentration of the urine. The total acidity in these patients varied from 68 to 80 and the  $p_H$  varied from 5.8 to 5.2.

In determining the acidity of the urine it is considered that an estimation of the hydrogen-ion concentration gives a better indication than the titration methods.

Hawk, in discussing urinary acidity, states that the acidity of the urine as determined by titration is not a good measure of the true acidity, which is dependent upon the concentration of hydrogen ions. In estimating the hydrogen-ion concentration in these cases and in the experiments carried on, the method of Medalia was followed. This method is simple, easy of application and gave very satisfactory results.

Hawk states that the normal value for the hydrogen-ion concentration of urine lies between 4.80 and 7.50 with a mean value of almost 6.00.

Starting on the assumption that an acid urine is necessary for the liberation of formaldehyde, and that this is the irritant factor, an effort was made to determine whether or not a highly acid urine was the deciding factor in the production of the hematuria following the administration of hexamethylenamina.

With this object in view four individuals were selected as controls, who showed a rather constant high acidity of urine. The urine was collected at three-hour intervals during the day, beginning at 7 A.M. and the hydrogen-ion concentration determined. On the third day hexamethylenamina was administered to these controls in doses of one gram four times a day dissolved in *Mistura Glycyrrhizæ Composita*. This was used as the solvent to ascertain, if possible, if it had any synergistic action in the production of a hematuria. Chart I shows the results of this experiment. The urine in all these cases was well below a  $p_H$  of 6 when hexamethylenamina was started. The administration of this drug was continued for a period of eight days without the production of a hematuria in any of the cases.

There was no change in the diet of these patients either before or during the experiment. It is interesting to note that the acidity of the urine as deter-

mined by the hydrogen-ion concentration was not increased by the administration of hexamethylenamina, as is inferred by some writers. In fact, the acidity of the urine was decreased in all of the four cases following the administration of hexamethylenamina.

From this experiment we were led to believe that some other factor beside a high acidity of the urine must be present in order to produce a hematuria. This experiment would also indicate that the *Mistura Glycyrrhizæ Composita* administered with the hexamethylenamina did not increase the power of the latter drug to produce a hematuria.

The presence of formaldehyde in the urine during this experiment was determined in each specimen by the Burnam test. This showed that the administration of one gram of hexamethylenamina four times a day showed a prac-

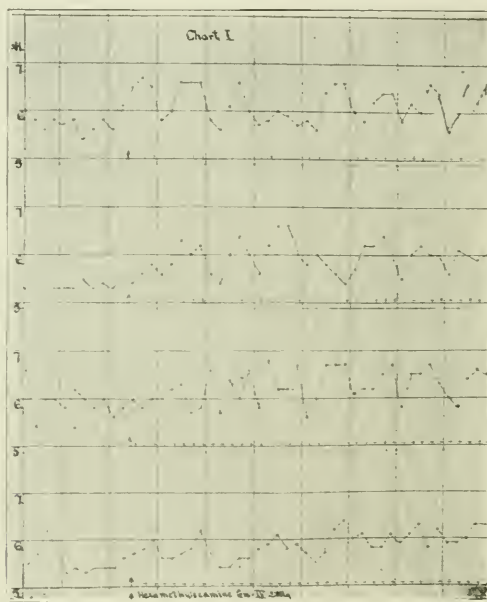


Chart I.—Showing the effect of the administration of hexamethylenamina on the pH of urine. Arrow indicates the beginning of medication. (Plus signs indicate positive Burnam test.)

tically constant positive Burnam test. The presence of a positive Burnam test in Chart I is indicated by a plus sign in each instance.

However, in order to bring out the factor of a high urinary acidity in the production of a hematuria more strongly, we conducted a second experiment using four individuals as controls and increasing the acidity of the urine to a greater degree than was present in the first experiment by the administration of acid sodium phosphate in conjunction with the hexamethylenamina. The results of this experiment are shown in Chart II. Four individuals were selected and were each given one gram of hexamethylenamina and two and a half grams of acid sodium phosphate four times a day. The  $p_H$  of the urine in these cases was kept well below 6, the acidity being constantly higher than in the first experiment. The administration of these drugs was continued for a period of eight days and in no instance was it possible to produce a hematuria.



This would indicate even more strongly than the first experiment that in order to produce a hematuria, some other factor must be present besides a high urinary acidity. The presence of a positive Burnam test on Chart II is indicated by a plus sign. The Burnam test was positive in practically every instance.

A third experiment was carried out in order to show the effect of the administration of sodium bicarbonate in conjunction with hexamethylenamina. For this experiment four individuals were selected and were each given one gram of hexamethylenamina and two and a half grams of sodium bicarbonate four times a day. Chart III shows the results of this experiment. The administration of these drugs was continued for a period of eight days without the

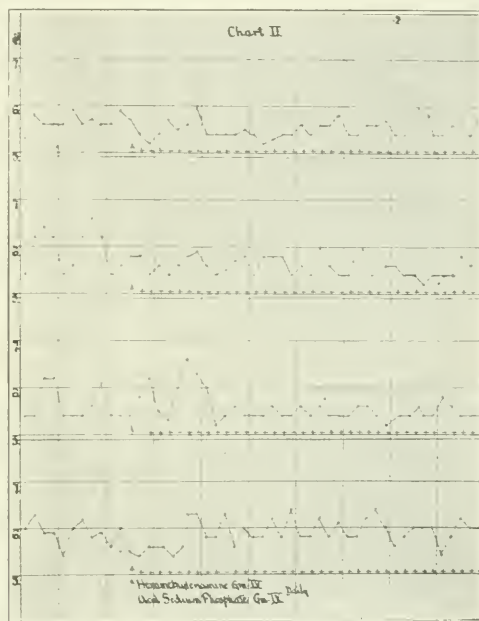


Chart II.—Showing the effect of the combined administration of hexamethylenamina and acid sodium phosphate on the pH of urine. (Plus signs indicate positive Burnam test.) Arrow indicates beginning of medication.

production of any untoward symptoms. A Burnam test was carried out on each specimen of urine and the  $p_H$  also determined. The hydrogen-ion concentration of the urine in these cases was definitely lowered and the  $p_H$  as a rule was well above 6, and showed a tendency to approach 7. A positive Burnam test in these cases is indicated on the chart by a plus sign, and this experiment would indicate that the administration of the above amount of sodium bicarbonate is sufficient to prevent the liberation of formaldehyde in the urine in almost every instance. In the few instances in which a positive Burnam was obtained this always occurred in the morning, and was accompanied by an increase in the hydrogen-ion concentration, the  $p_H$  in each instance being well below 6.

This experiment would tend to show that an acid urine is necessary for the production of a hematuria following the administration of hexamethyl-

enamina, but that in addition to this acidity of the urine some other factor must be present.

Chart IV shows the hydrogen-ion concentration of the urine in the first four cases of hematuria reported in this series. Case V was not available for this study. This chart showed that the  $p_H$  of these cases on ordinary diet as a rule was well below 6. The urine of these cases was collected at intervals of one hour and the  $p_H$  immediately determined. At the time of conducting this experiment these patients were convalescing from their hematuria.

The results of the observations on these cases and the experimental data obtained so far may be summed up as follows:

Following the administration of hexamethylenamina in doses varying

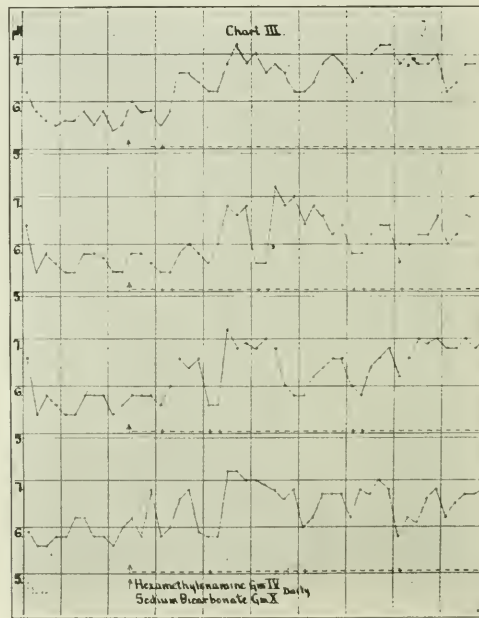


Chart III.—Showing the effect of combined administration of hexamethylenamina and sodium bicarbonate on the  $p_H$  of urine. (Plus signs indicate positive Burnam test.) Arrow indicates beginning of medication.

from 30 to 60 grains daily to approximately four hundred cases, there developed five cases of hematuria. Each of these cases showed a high hydrogen-ion concentration of the urine. However, this in itself is not considered sufficient to produce a hematuria. We are led to believe from this data and experiments conducted that for the production of a hematuria following the administration of hexamethylenamina an idiosyncrasy for this drug must be present.

In an attempt to demonstrate an existing idiosyncrasy we employed a skin reaction test. For this purpose dilutions of the drug of 1 to 5, 1 to 10, 1 to 50 and 1 to 100 were employed. A 1 to 50 solution of formalin was also used. As control solutions caffeine 1 to 10, salicylic acid 1 to 10, quinine chlorhydrosulphate 1 to 20, adrenalin 1 to 1000 and normal saline were used.

Twelve individuals were used as controls to whom hexamethylenamina had been administered in doses of 1 gram four times a day for a period of eight days, and who showed no untoward symptoms following the administration of this drug. The flexor surface of the forearm was used for the tests and the skin scratched with a needle sufficiently to produce an abrasion but not to cause bleeding and the solutions of the various drugs were rubbed into the scratch marks. The controls in each case gave negative results, while a positive reaction was noted in each of the five hematuria cases, which was most pronounced in the 1 to 5 and 1 to 10 dilutions of hexamethylenamina. This reaction reached its maximum in 15 to 20 minutes following the applica-

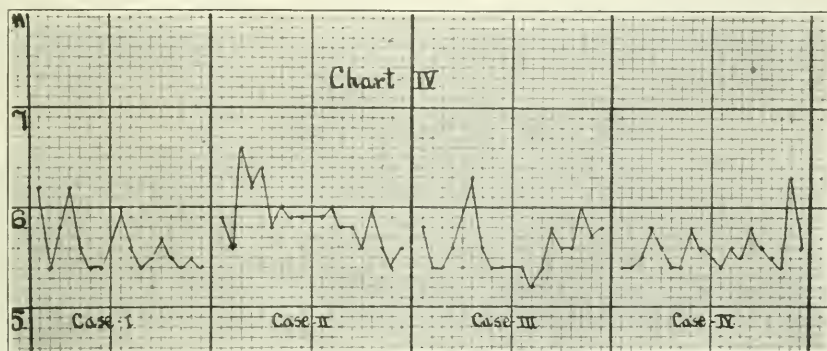


Chart IV.—Showing the PH of the urine of first four cases during convalescence from hematuria.

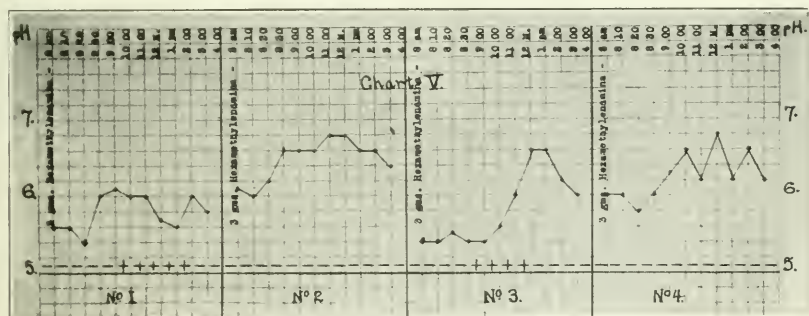


Chart V.—Showing time of appearance of formaldehyde in urine after administration of 3 gms. of hexamethylenamina and the PH of the urine. (Plus sign indicates positive Burnam test.)

tion of the drug, and consisted in redness, induration and the production of a raised area which is shown in the accompanying photographs. The application of the formalin solution caused a slight local reaction.

The cases which showed the most marked hematuria and in which the symptoms of irritation were most pronounced also gave the most marked skin reaction. Figs. 1, 2, and 3 show the type of reaction obtained in Case III and Case V.

In Case III skin tests were continued at intervals of about one month for four months after discharge from hospital. It was found that the tendency was for these tests to become less marked on each occasion, although at the end of the third month the test was still positive.

We also performed a skin test on an individual who gave a history of hematuria following the administration of hexamethylenamina eight years previously, and obtained a negative result. Whether the sensitiveness to hexamethylenamina decreases and disappears entirely, or, in other words, whether it is merely a temporary or a permanent sensitiveness, appears somewhat uncertain, but it is not unlikely that in these two cases the patients may have developed an acquired immunity, at least to some extent.

The results in these cases have been sufficiently encouraging to warrant the opinion that individual hypersusceptibility to hexamethylenamina is a contributing factor in the production of hematuria. Whether cutaneous hypersensitiveness should be placed in the domain of anaphylaxis or merely drug idiosyncrasy, we have insufficient evidence on which to base a positive con-

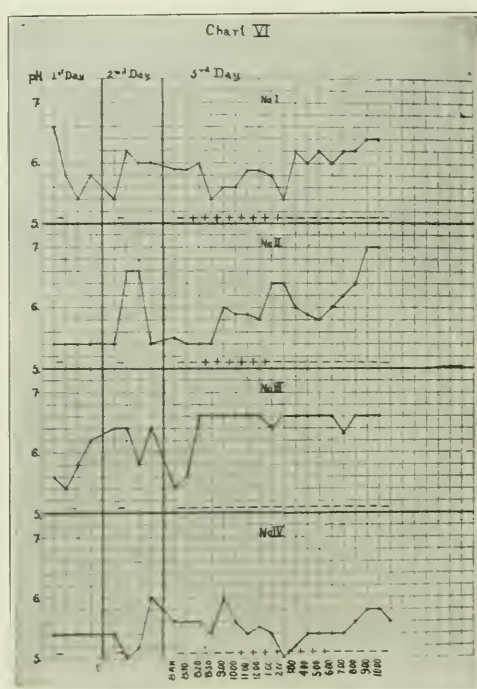


Chart VI.—Showing time of appearance of formaldehyde in urine after the administration of acid sodium phosphate. 3 gms. hexamethylenamina administered at 8 A.M. the third day. (Plus sign indicates positive Burnam test.)

clusion. However, there seems to be much in common in their symptomatology, and the differential diagnosis of drug and food idiosyncrasy and allergy is not easy.

Several hypotheses are advanced as to the necessary mechanism for the production of cutaneous reaction to drugs and other substances not containing proteins. Park states that we have no evidence that the basis of these reactions is an antigen-antibody reaction, a hypothesis, which seems to be required in specific anaphylaxis, and he considers the symptoms elicited as due to idiosyncrasy. On the other hand, Kolmer and others believe that these are true anaphylactic skin reactions due to the interaction of a specific anaphylactic antibody and



specific anaphylactogen largely within or without the cells, and with the formation of diffusible irritants capable of producing acute hyperemia, edema and leukocytic infiltration of the skin.

The fact remains that from our observations the evidence is fairly conclusive that there exists in these individuals a susceptibility to hexamethylenamina which could not be demonstrated in the controls. Furthermore, with the evidence already at hand that it was not possible to produce hematuria in unsusceptible individuals, taking the hyperacid urine as a necessary factor in the elaboration of the irritant formaldehyde as the contributing cause, we have come to the conclusion that the evidence is sufficient to warrant the statement that individual hypersensitivity or an allergy to hexamethylenamina is the etiologic factor in the production of these hematurias.

The source of the blood in hematuria caused by the administration of hexamethylenamina has been more or less indefinite. The prevailing opinion



Fig. 1.—Showing local reaction from various drugs inoculated into skin abrasions on forearm in the following dilutions: hexamethylenamina 1:5, 1:10, 1:50, 1:100; caffeine citrate 1:10; salicylic acid 1:10; quinine chlorhydrosulphate 1:20; adrenalin 1:1000; normal saline (0.85%); formalin 1:50.

seems to be that it comes from the bladder and that the kidneys remain unaffected.

The evidence obtained in the above five cases of hematuria points fairly definitely to the bladder as the site of the hemorrhage. The presence of an acute hemorrhagic nephritis which would be necessary to cause such a marked hematuria of renal origin, could be ruled out by the absence of constitutional symptoms, the absence of casts, especially blood casts, in the urine, the rapid subsidence of the symptoms and the complete recovery of the patient in a few days with no sequelæ. The symptoms in these patients pointed directly to the bladder as evidenced by the tenesmus, vesical pain, marked irritation and the terminal hematuria. And finally, the definite lesions noted on cystoscopic examination leave little room for doubt regarding the source of the hematuria following the administration of hexamethylenamina.

These lesions consist of hemorrhagic areas, irregularly distributed over the base of the bladder, about the ureters and neck of the bladder, varying in size from  $\frac{1}{2}$  to 2 cm. in diameter and appearing bright red in color.

In an effort to determine the time necessary for the appearance of formaldehyde in the urine following the administration of hexamethylenamina, four individuals were selected and were each given 3 gms. of hexamethylenamina at 8 A.M. Chart V shows the result of this experiment. A positive Burnam test is indicated on this chart by a plus sign. The  $p_H$  of the urine was determined on each specimen examined.



Fig. 2.—Showing local reactions from various drugs inoculated in skin abrasions on forearm in the following dilutions: hexamethylenamina 1:5, 1:10, 1:50, 1:100; caffeine citrate 1:10; salicylic acid 1:10; quinine chlorhydrosulphate 1:20; adrenalin 1:1000; normal saline (0.85%); formalin 1:50.



Fig. 3.—(K) Showing local reactions from various drugs inoculated into skin abrasions on forearm in the following dilutions: hexamethylenamina 1:5, 1:10, 1:50, 1:100; caffeine citrate 1:10; salicylic acid 1:10; quinine chlorhydrosulphate 1:20; adrenalin 1:1000; normal saline (0.85%); formalin 1:50.

In Number I a positive Burnam was obtained two hours following the administration of hexamethylenamina. The  $p_H$  of the urine in this case remained well below 6 until 8:30 A.M., when it reached 6, at which point it remained for two and a half hours. The positive Burnam test persisted for four hours in this case.

In Number II the  $p_H$  of the urine was well above 6 and at no time was

a positive Burnam obtained following the administration of hexamethylenamina in this case.

In Number III a positive Burnam was obtained one hour following the administration of hexamethylenamina. In this case the  $p_H$  of the urine remained well below 6 for three hours following the administration of the drug. The positive Burnam persisted for three hours.

In Number IV the  $p_H$  of the urine showed a tendency to stay well above 6 and a positive Burnam was not obtained.

This chart emphasizes very strongly the importance of the acidity of the urine in the liberation of formaldehyde after the administration of hexamethylenamina.

In an effort to bring out this point more strongly, four individuals were selected and placed on acid sodium phosphate gms. 4, three times a day. On the morning of the third day they were each given 3 gms. of hexamethylenamina at 8 A.M. Chart VI shows the result of this experiment.

In Number I the  $p_H$  of the urine was kept fairly constantly below 6 and a positive Burnam test was obtained twenty minutes following the administration of the drug, and persisted for five and a half hours.

In Number II the  $p_H$  of the urine was well below 6 on the third day and a positive Burnam was obtained thirty minutes after the administration of hexamethylenamina, which persisted for four and a half hours.

In Number III the  $p_H$  of the urine showed a tendency to remain well above 6 and a positive Burnam was not obtained.

In Number IV the  $p_H$  of the urine was kept well below 6 and a positive Burnam was obtained three hours following the administration of the drug and persisted for seven hours.

Charts V and VI indicate that a  $p_H$  below 6 is necessary for the liberation of formaldehyde in the urine following the administration of one dose of 3 gms. of hexamethylenamina. In cases in which the  $p_H$  of the urine remains constantly above 6 a positive Burnam is not obtained following the administration of this amount of the drug.

Where the urine is sufficiently acid, a positive Burnam test is obtained in from twenty minutes to three hours following the administration of the drug.

In an effort to obtain more information regarding the pathology of hematuria caused by hexamethylenamina, it was decided to attempt some experiments with the guinea pig. For this purpose a preliminary experiment was performed in feeding hexamethylenamina to guinea pigs in moderate doses, and it was found that a hematuria could easily be produced with this drug.

For the final experiment, six guinea pigs were selected of an average weight of 500 gms. and the urine of these pigs examined for several days to rule out a preexisting hematuria. When this had been definitely ruled out the administration of the drug was commenced, beginning with a daily dose of 20 mg., half of which was administered in the morning and half in the evening. Table I shows the complete report of this experiment. It appears





TABLE I—CONTINUED.

[illegible]

TABLE 1—CONTINUED.

Chart showing effects of administration of Hexamethylenamina to guinea pigs. — indicates no red blood corpuscles in urine. \* indicates presence of red blood corpuscles in urine microscopically. \*\* indicates numerous red cells in urine. \*\*\* indicates frank hematuria with blood present macroscopically in urine. K indicates killed. D indicates died.

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14 to 22	23	24 to 30	POSTMORTEM FINDINGS
Hexamethylen- amina in mg.	20	20	20	20	20	20	20	40	40	80	80	80	80	80	80	80	
Guinea Pig No. 5	-	-	-	-	-	**	**	**	***	***	***	***	***	***	*** K		<p><i>Kidney:</i> Extensive acute congestion, slight acute parenchymatous degeneration. <i>Intestines:</i> Marked congestion involving the mucosa and the submucosa. <i>Liver:</i> Acute congestion marked. <i>Uterus:</i> Congestion. <i>Bladder:</i> Acute congestion involving mainly the submucosa. <i>Adrenal:</i> Very slight congestion subcapsular and medullary.</p>
Guinea Pig No. 6	-	-	-	-	-	**	***	***	***	***	***	***	***	***	***	***	Drug discontinued. Recovery.

from a study of this table that a daily dose of 20 mg. of hexamethylenamina to guinea pigs will produce a hematuria in from two to seven days. The drug is certainly toxic for these animals and their deterioration in health and general appearance is quite noticeable if the drug is continued. It appears, however, that the lesions in the guinea pig are fairly widespread, and there is definite evidence of acute inflammatory processes involving the genitourinary tract, the gastrointestinal tract and the abdominal viscera. In the case of the guinea pig the hematuria is undoubtedly renal in origin, although it is quite likely that the whole genitourinary tract contributes to the hematuria as well as the kidney.

To further implicate the kidney as the source of the hematuria in the guinea pig, it was decided to tie off the ureter in a pig in which hematuria had been established by the administration of hexamethylenamina. It was found in this case that the urine collected in the kidney pelvis was distinctly hemorrhagic, and it is believed that this evidence, in conjunction with the postmortem findings, leaves no room for doubt regarding the toxic effect of hexamethylenamina on the kidneys of the guinea pig.

We are indebted to the U. S. Naval Medical School, Washington, D. C., for the pathologic reports on the guinea pigs.

However, the picture of hematuria in man is considerably different from that in the guinea pig, although if enormous doses were given to man and continued over sufficient periods, it appears quite likely that a definite nephritis could be produced.

#### SUMMARY

Five cases of hematuria developed among approximately four hundred cases of mild influenza who received from 30 to 60 grains a day of hexamethylenamina.

The hematuria in these patients appeared in from one to seven days following the administration of the drug. All of these patients showed a high hydrogen-ion concentration of the urine. In an attempt to demonstrate an idiosyncrasy to hexamethylenamina in these patients, the drug was applied in varying dilutions to the slightly abraded skin of the forearm and in each case a reaction consisting of redness, induration and the production of a raised area, was noted.

The urine showed no blood casts; there were no constitutional symptoms and no sequelæ. The bladder showed definite hemorrhagic lesions through the cystoscope. These hemorrhagic areas were irregularly distributed over the base of the bladder and about the ureters, the vault and lateral surfaces being unaffected.

Attempts to produce hematuria in individuals who showed a high hydrogen-ion concentration of the urine were unsuccessful. Even when the  $p_H$  of the urine was kept low by the administration of acid sodium phosphate it was impossible to produce hematuria when hexamethylenamina was given in doses of gms. 4 daily for eight days.

The administration of sodium bicarbonate gms. 10 daily in conjunction with

the hexamethylenamina served to prevent the liberation of formaldehyde in the urine in almost every instance.

The Burnam test was used to demonstrate the presence of formaldehyde in the urine. No doubtful reactions were reported and in every instance when a positive result was reported the reaction was unmistakable.

In individuals in which the  $p_H$  of the urine was below 6 a positive Burnam test was obtained in from twenty minutes to three hours following the administration of gms. 3 of hexamethylenamina and persisted for from three to seven hours. In individuals in which the  $p_H$  of the urine remained above 6 a positive Burnam was not obtained following the administration of 3 gms. hexamethylenamina.

The drug is toxic to guinea pigs and in doses of 20 mg. a day produced a hematuria in from two to seven days. The lesions in the guinea pig are widespread and there is definite evidence of acute inflammatory processes involving the genitourinary and gastrointestinal tract with marked acute nephritis and acute congestion of the bladder. The hematuria in the guinea pig is of renal origin, although the bladder may also act as a contributing source.

#### CONCLUSIONS

From a study of these cases of hematuria and from the experiments conducted it is believed that we are justified in drawing the following conclusions:

1. Following the administration of hexamethylenamina in even moderate dosage a small percentage of individuals will develop hematuria.
2. These cases show a high hydrogen-ion concentration of the urine.
3. It is considered that these individuals possess an idiosyncrasy to hexamethylenamina.
4. This idiosyncrasy can be demonstrated by a cutaneous reaction test, using the drug itself as the antigen.
5. A high hydrogen-ion concentration favors the elimination of formaldehyde in the urine and appears to be a necessary factor in the production of hematuria.
6. A high hydrogen-ion concentration of the urine is of itself not sufficient to cause a hematuria following the administration of hexamethylenamina.
7. The hematuria following the administration of hexamethylenamina in individuals is vesical in origin.
8. The lesion consists of a hemorrhagic cystitis involving the base and neck of the bladder; the hemorrhagic areas are irregularly distributed and when viewed through the cystoscope present a fairly typical picture.
9. Attempts to produce hematuria in unsusceptible individuals by the administration of hexamethylenamina were unsuccessful even when the  $p_H$  of the urine was kept low by the administration of acid sodium phosphate.
10. The administration of sodium bicarbonate in doses of 10 grams daily in connection with hexamethylenamina will prevent the liberation of formaldehyde in most instances.



11. When the urine is sufficiently acid a positive test for formaldehyde can be obtained in from twenty minutes to three hours following the administration of 3 grams of hexamethylenamina.

12. When the  $p_H$  of the urine is much above 6 the liberation of formaldehyde is interfered with.

13. A  $p_H$  as low as 6 is impossible in any other medium in the body except the urine and the gastric juice, and if hexamethylenamina is dependent on the liberation of formaldehyde for its antiseptic properties, these properties can never be manifested except in the genitourinary tract.

14. Hexamethylenamina is toxic for guinea pigs and produces extensive inflammatory reactions in the genitourinary and gastrointestinal tracts.

15. The hematuria in guinea pigs is chiefly renal in origin.

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## CHRONIC URTICARIA OF FIVE YEARS' DURATION PROBABLY DUE TO CHRONIC ACIDOSIS\*

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THE following very severe chronic and intractable case of urticaria appears to me to present enough points of interest to justify a somewhat detailed report and discussion.

The case, No. 2060, Mr. G., an attorney, age forty-five, consulted me on June 15th, 1920, because of a skin lesion of five years' duration, which on examination proved to be a typical urticaria universalis, which had begun on legs and extended to entire body. Every night during this five-year period the patient was kept awake the greater part of the time by the associated severe pruritus. He had been under treatment the greater part of this period without obtaining relief. The wheals varied in size from 2 to 10 cm. in diameter. He would be comparatively free from symptoms during the day, but they invariably returned during the early part of the night and tortured him until morning. His general health had suffered very severely because of interference with rest and sleep and because of an accompanying diarrhea contracted in subtropical regions one year preceding the onset of the "hives", and which symptom had troubled him more or less ever since. The obvious bearing of this incident was fully appreciated as will be seen later. The patient also complained of irritability, marked general debility, slight dyspnea on exertion, and some discomfort with gas distention after eating.

The family history was entirely negative. There was nothing important in the past history excepting severe scarlet fever at age of nineteen, followed by "kidney trouble and dropsy", from which he apparently made a complete recovery.

On physical examination he presented a worried and fatigued expression, fairly well nourished, weight 141 pounds, about ten pounds below his normal weight; pulse 82, T. 98.8. R. 22; blood pressure, systolic 110, diastolic 58; a well marked taché, indicating vasomotor instability, which corresponds with the large pulse pressure above shown. The physical examination of thorax and abdomen was entirely negative with the exception of the hives. The laboratory findings were as follows: Urine entirely negative, specific gravity 1016-1030; blood: red cells 5,500,000, white blood cells 5,700, hemoglobin 92 per cent, differential, Polys. 57 per cent, S. L. 10 per cent, L. L. 13 per cent, Trans. 1 per cent, Eos. 19 per cent, (28 per cent a little later). Wassermann negative. CO<sub>2</sub> combining power of plasma 50+ volumes per cent; blood no procurable in fasting condition. Fees: On account of eosinophilia and patient's former residence in the subtropical region, four exhaustive studies were made, especially for blood and for ameba and other parasites and ova, by warm stage, but with entirely negative results. Gastrointestinal: Ewald's test meal:

1	hr.p.c.	Free HCl	42,	Total	54
1½	hr.p.c.	"	"	62,	" 72

no mucus, no occult blood. Roentgenologic study of barium meal showed perfectly normal position, mobility, motility and contour of the gastrointestinal tract, with the possible exception of a moderate deformity of the duodenal cap, suggesting an old lesion. The stomach

\*Received for publication, October 24, 1921.

†This alkali reserve work was done by the Marriott method before it was discarded for the more accurate Van Slyke. The findings are expressed comparatively in terms of CO<sub>2</sub> combining power of plasma.

was entirely empty in four and one half hours. Serial plates revealed no pathology. Fluoroscopic palpations disclosed no adhesions anywhere.

In a careful study of the data above recorded, the low alkali reserve appeared to be one of the most important clinical findings. The patient was therefore placed upon sodium bicarbonate, 8 grams per day tentatively, with an exclusive diet of milk, cream and cereals, and a borax bath once a day. This quantity of sodium bicarbonate failed to produce a neutral urine and in a few days the dose was doubled giving him 16 grams daily with orders to vary the daily intake of the sodium bicarbonate so as to produce a urine reaction neutral or alkaline to litmus, allowing it to become slightly acid once in 24 hours. At the end of one week there was no improvement. In two weeks he reported entire freedom from urticaria for 48 hours, the first time in five years. Improvement from this time was rapid, and in about another week the symptoms disappeared entirely and only recurred in a very light form twice since then for a single night, each time coincident with a "cold."

The patient has now been entirely free from urticarial lesions and symptoms for about 15 months. His general condition has correspondingly improved. He has gained nine pounds in weight and says that he is in better health than in a great many years.

During the progress of the treatment several very interesting changes took place in laboratory findings. On Sept. 10, 1920, and Oct. 6, 1920, the  $\text{CO}_2$  combining power of plasma was 66 volume per cent. The eosinophilia which was an interesting and conspicuous feature of the case gradually became less and less. On Oct. 6, 1920, it had fallen to 8 per cent, and on Dec. 13, 1920, in a count of 100 cells no eosinophils were found.

Perhaps the most interesting observation made in the study of this case was the low alkali reserve indicating a moderate grade of chronic acidosis which was confirmed by the large amount of sodium bicarbonate required to neutralize the urine. This is of special importance because of the recent simplifications of technical methods of blood examinations which make it possible for the clinician to procure information of this sort with sufficient accuracy to meet the requirements of clinical diagnosis and subsequent treatment.

The problem of acidosis is too large to justify an attempt at its full discussion here. Briefly it may be said that the determination of the  $\text{CO}_2$  combining power by the Van Slyke method is a means of estimating the reduction in the quantity of so-called "alkaline buffer substances" in the blood, namely sodium bicarbonate and disodium phosphate; this reduction in the buffer substances occurring because of their neutralization by non-volatile acids, which, unlike the  $\text{CO}_2$ , cannot be eliminated through the lungs. They are permanently fixed by the acid and are therefore functionally lost to the organism, and to this extent these alkali reserve substances are reduced. These alkaline bodies are the "normal carriers" of  $\text{CO}_2$  from tissues to lungs where this volatile acid is eliminated. When the capacity of the blood for carrying  $\text{CO}_2$  from the tissues to the lungs is reduced, acidosis results. Under normal conditions for adults at rest the  $\text{CO}_2$  combining power of blood plasma

is 77 to 53 c.e. per 100. As the reserve alkali is used up more and more by combination with the nonvolatile acids, the  $\text{CO}_2$  combining capacity is diminished to from 53 to 31 c.e. per 100, the lower figure, and figures ranging occasionally even lower than this, applying to the most severe cases of acidosis.<sup>1</sup>

Now what relation, if any, can this acidosis be safely assumed to have to the urticaria in the case under discussion? The answer to this question is that it is probably indirect and depends upon a number of facts, some of which are well established and others conjectural.

What is the real nature of urticaria? Sutton<sup>2</sup> says that it is very probably always due to anaphylaxis. Darier<sup>3</sup> is not so positive and says that the majority of cases of urticaria are due to anaphylaxis.

Now if we are to assume an anaphylactic origin in this case, obviously the diet question must receive careful consideration. This patient was placed at the outset upon the exclusive diet of milk, cream and cereals, and it is entirely possible that the anaphylactic substance, if such exists, might have been removed by the sweeping reduction of diet which eliminated all meats and vegetables. From this promiscuous list a considerable proportion, most anaphylactic substances of dietetic origin, are probably derived. It does not seem that we can come closer than this to the answer to the question of dietetic anaphylactic substances as etiologic factors in this case. There is, however, another suggestion along the line of anaphylaxis which is derived from the fact that the only two slight recurrences of urticaria which he has had occurred in connection with "colds." This naturally suggests the possible operation of bacterial anaphylaxins.

With reference to the etiology of the acidosis we must carefully consider intestinal conditions. The history clearly indicates perverted digestion, with gas formation, slight discomfort with a moderate hyperchlorhydria. This perverted digestion might be fairly assumed to increase the production of nonvolatile acids in the lower segments of the small intestine. The fact is that the contents of the small intestine are more acid than formerly supposed, and that this acidity is due to fixed acids, largely of the fatty type. It has been shown that certain bacterial enzymes under favorable conditions may transform carbohydrates into lactic acid, and this acid may by further action of similar enzymes be converted into butyric acid.<sup>4</sup> There are also certain lipolytic enzymes of bacterial origin which may split up the fats with the production of valeric, butyric and other lower acids.<sup>5</sup> It has been demonstrated that butyric and other fatty acids can be transformed into aceto-acetic acid<sup>6</sup> and there is no reason to doubt that these transformations may and do occur within the intestinal tract. In addition it has been shown that aceto-acetic acid can be produced from the amino-acids by protein cleavage.<sup>7</sup> As a matter of course, of the other two members of the ketonic group, acetone can be produced by oxidation of aceto-acetic acid and the beta-oxybutyric by its reduction. So far as this particular group is concerned we have a perfectly plausible source in intestinal fermentation from which they may enter the circulation in quantities sufficiently large to combine with reserve alkalies



to an extent which might seriously deplete the latter. These bodies are dwelt upon with special emphasis, largely because of the conspicuous rôle they play in diabetic and other types of acidosis. It must be borne in mind that other acids, some of which are mentioned above, may lead to the formation of these bodies, thus becoming as easily available and effective in the production of acidosis.

In considering the etiologic relationship of the acidosis to the urticaria in this case, conservatism is suggested by the fact that such clinical presentations are relatively rarely seen, even in cases of severe acidosis, especially the diabetic type on the verge of coma. While the etiologic factors in this case may not be clearly proved, the prompt clinical result of treatment based upon a low alkali reserve with a single addition of a simplified diet, is certainly of striking interest.

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## STUDIES IN POSTMORTEM BACTERIOLOGY: VALUE AND IMPORTANCE OF CULTURES MADE POSTMORTEM\*

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IT has long been recognized that the greater number of all deaths are the result of primary or secondary invasion of the body by pathogenic bacteria. Fortunately for the clinician and pathologist, most of these organisms leave a fairly characteristic trail, and the reactions between them and the living tissues are so specific that, within certain limits, a careful study makes a presumptive diagnosis moderately certain. However, in at least two circumstances the accuracy of such a clinical diagnosis often becomes questionable: (1) if the reaction of the body is rendered atypical either from unusual variation in immunity, or in the virulence of the invader, the identity of the offending organism is obscured, and (2) if the original focus of the parasite is hidden and the absorbed toxin is extremely destructive, clear proof of the identity of the organism is obscured.

So serious are the results of errors of diagnosis that modern clinical practice demands careful bacteriologic studies as controls in all cases of suspected infection. To mistake the lesions of diphtheria for those of tonsillitis, or the twitching of tetanus for those of tetany are indefensible errors unless all the facilities of modern bacteriology have been exhausted.

For the pathologist, bacteriology is equally important, but it is too often neglected or it is used only superficially or incompletely. The two sources of error to which clinicians are exposed are even more prominently emphasized at the postmortem table, where signs are few, symptoms entirely absent, and appearances often deceiving. Moreover, the lesions which have finally caused death are not always sufficiently evident to be recognized even with the aid of the microscope. In such cases the presence or absence of pathogenic bacteria becomes an absolutely decisive factor. Even in lesions in which it becomes clearly apparent that bacteria are largely responsible, the species or type of organism is not demonstrable without appropriate technical procedure.

Two fundamental propositions are established for the clinician and the pathologist: (1) every infection requires the accurate identification of the offending agent, and (2) in every obscure condition, the presence of pathogenic bacteria must be excluded by systematic routine bacteriologic methods before a definite conclusion may be reached. Carelessness in this respect can too often be charged against the pathologist. Indeed the meager reports in the literature on this subject indicate a disregard of this fundamental practice.

Postmortem bacteriologic examinations have been declared unreliable by certain workers. Gradwohl, in a study of fifty cases, came to the conclusion

\*These studies were made in the Section on Pathologic Anatomy, Mayo Clinic, at the suggestion and under the supervision of Dr. H. E. Robertson.

Received for publication, Nov. 4, 1921.

that invasion from neighboring organs occurs, either agonal or immediately after death. Gwyn and Harris, in a comparative study of the results of blood cultures made during life and after death, concluded that an organism in a local lesion grows and spreads in the blood stream after death. Buxton has shown that typhoid bacilli introduced into the peritoneal cavity of living animals may be taken up almost immediately to be deposited in various organs. That this occurs during life suggests the fallacy of assuming agonal or postmortem invasion in bacteremias associated with peritonitis.

On the other hand equally careful workers regard postmortem bacteriologic examinations worthy of serious consideration. Fredette believes that cultural results obtained a few hours after death are fairly reliable in demonstrating the presence of organisms existing at the time of death, although he admits the possibility of postmortem invasion. Canavan and Southard, in a series of cultures in 200 cases postmortem, concluded that their findings pointed definitely to the intravital occurrence of the bacteria found, although in no instance was the chain of evidence complete. They further noted that the percentage of positive cultures did not differ significantly at varying hours after death so that the danger of contamination may be limited to a brief interval. Gwyn and Harris conclude, "Bacteremia, due to common organisms of the intestinal tract and the so-called agonal invasions of the blood stream, do occur, yet they should not be assumed to be present with any great degree of frequency."

It is obvious that the opinions of the workers concerning the postmortem invasion of bacteria are divided. Accordingly our investigation was made for the purpose of determining two questions: Does invasion occur postmortem, and, if so, within what limits? Do bacteria recovered at postmortem have any intravital significance?

Scrupulous attention to details of technic is necessary at all stages of the experiment. The organ to be cultured is seared with a red hot spatula, and care is exercised to prevent liquids or melted fat from the adjacent tissues from contaminating the seared surface. A sterile pipette of approximately 5 c.c. capacity, kept in a sterile test tube, is thrust through the seared surface and the desired amount of material aspirated. Solid organs are pierced at such an angle that the point of the pipette will be in the depth of the organ and away from the superadjacent seared surface. The material thus collected is immediately transferred to a tube of glucose-brain broth prepared according to the method of Rosenow. If these cultures are positive, subcultures on blood agar plates and other mediums are made. A portion of a small amount of exudate or of material from a given organ is spread over a blood agar plate and another portion is spread on a slide to be stained later.

There are several reasons for selecting glucose-brain broth as a culture medium. It is inexpensive and easily prepared. As Rosenow has pointed out, "The bottom of the tube is rendered anaerobic while the top necessarily remains aerobic and the space between represents a gradient of oxygen pressure intermediate between the two extremes." He has further observed, "The infecting organism may be sensitive to oxygen pressure." In this man-

ner bacteria that are sensitive to slight changes in oxygen pressure may be grown. We have been able to grow strict anaerobes in this medium.

In our series of 213 cases the blood and spleen were cultured routinely; the spleen was cultured in order to determine if it acted as a bactericidal factor or as a filter for organisms. Other systems, chiefly the genitourinary, respiratory, and cerebrospinal systems, were cultured as conditions demanded.

Bearing in mind the fact that infected foci may be the source of general infections, and, as many writers have pointed out, may be the cause of certain diseases, we cultured teeth which were condemned by dental examinations made before death.

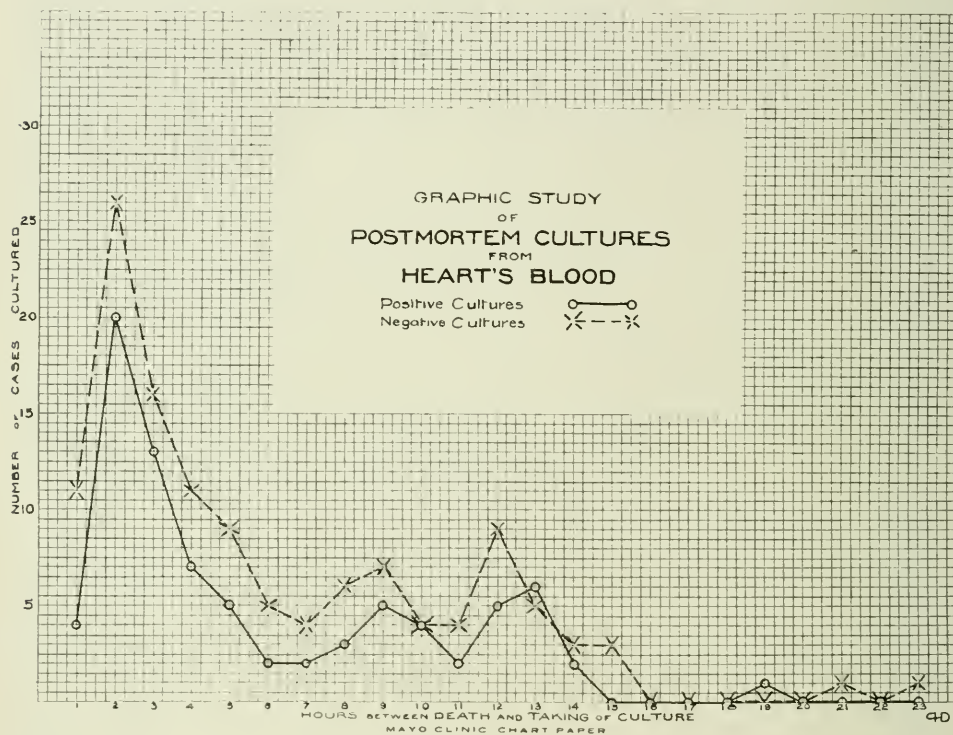


Fig. 1.

The relative pathogenicity of organisms recovered and their significance as the cause of certain lesions can best be established by animal inoculation. We have therefore resorted to this procedure in obscure cases, using rabbits and other laboratory animals. The bodies used were not subjected to refrigeration, but were exposed to the temperature prevailing in summer or winter and for the periods of time indicated in Fig. 1.

Gwyn and Harris have compared antemortem and postmortem cultures in fourteen cases with a view to determining the reliability of postmortem cultures. In seven cases the agreement is absolute; two cases show the presence of an additional organism after death due to a superadded terminal infection; and five cases show radical differences in antemortem and postmor-



tem results. Richey and Goehring report twenty-four cases in which both antemortem and postmortem cultures were made, in twenty of which the agreement is absolute. However, we cannot draw too sharp conclusions from this type of evidence, for antemortem cultures may be made several days before death, may precede the advent of an infectious process, or may be taken in a period of bacteremia which later disappears, all of which may account for disagreement between antemortem and postmortem cultures. In order to test this point we have tabulated in Table I the results in twenty-two cases of the series in which antemortem cultures had been made. In fifteen of the twenty-two cases the results were in agreement.

In Case 9, culture at death, five days after the culture before death, yielded a gas-producing anaerobic bacillus. That this organism was not obtained before death is probably owing to the unsuitable method of growing anaerobic bacteria. The postmortem result is further strengthened by the fact that the clinicians regarded the condition as septicemia.

In Case 4, antemortem culture antedated the postmortem culture by sixteen days. In the meantime the patient contracted bilateral bronchopneumonia, which may account for the positive culture obtained after death.

In Case 10, the positive culture obtained after death is substantiated by the postmortem findings, which included general and localized peritonitis and serofibrinous pleuropneumonia, evidences of a widespread infection. The negative antemortem culture is not invalidated, for it was taken nine days earlier and may have preceded the advent of the bacteremia.

In Case 1, five days elapsed between the cultures. Postmortem findings undoubtedly supported the positive bacteriologic findings. Necropsy revealed bilateral suppurative otitis media and mastoiditis, thrombosis of the right lateral and sigmoid sinuses, multiple pulmonary abscesses, bilateral suppurative mastitis, and empyema in the left side.

In Case 7, an interval of fourteen days separated the cultures made before and after death. The presence, at necropsy, of bilateral empyema, bilateral perirenal abscesses, bilateral pyelonephritis, and an abscess in the psoas muscle is indicative of a widespread infection and corroborates the cultural results obtained after death.

Case 14 presents a typical picture of septicemia before death, but two antemortem cultures made thirty-eight and thirteen days before death were negative. Necropsy revealed multiple pyemic abscesses of the extremities, vegetative endocarditis, thrombosis of both femoral veins, a large psoas abscess, and bilateral multiple focal abscesses of the kidneys, all of which attest the validity of the postmortem cultures.

The last of five negative cultures obtained before death in Case 3 was made eighteen days before death. In support of the positive cultures after death necropsy findings are invoked of acute general peritonitis following perforation of the colon, of acute bilateral pyelitis, of acute bilateral bronchopneumonia with pleuritis, and disseminated petechial hemorrhages and multiple subcutaneous purpuric spots.

In our experience it has not been necessary to attribute discrepancies

TABLE I  
COMPARISON OF ANTEMORTEM AND POSTMORTEM CULTURES

CASE	NECROPSY NUMBER	POSTMORTEM DIAGNOSIS	DATE OF ANTEMORTEM CULTURE	RESULT OF CULTURE	DATE OF POSTMORTEM CULTURE	LENGTH OF TIME AFTER DEATH, HOURS	RESULT OF CULTURE
1	409-20	Sinus thrombosis, bilateral otitis media	7-19-20	Negative	7-22-20	3	<i>Streptococcus hemolyticus</i> in the blood, spleen, and lung abscess
2	425-20	Pylonephritis, endocarditis	7-29-20	Negative	7-30-20	1	Negative
3	427-20	Ulcerative colitis, peritonitis	7-13-20	Negative	8-1-20	4	Blood, spleen, lung, and kidneys positive
4	455-20	Pneumonia, ulcerative stomatitis	7-31-20	Negative	8-16-20	4	<i>Streptococcus</i> in the blood, spleen and lungs
5	460-20	Thrombophlebitis	8-11-20	<i>Streptococcus</i> in blood	8-17-20	1	<i>Streptococcus hemolyticus</i> in the blood and spleen
6	485-20	Malignant endocarditis	8-28-20	<i>Streptococcus viridans</i> in blood	9-1-20	3	<i>Streptococcus viridans</i> in the blood, spleen, and vegetations of the endocardium and pleural fluid. <i>Bacillus coli</i> and streptococci in blood and kidneys.
7	559-20	Bilateral empyema, pyelonephritis	9-23-20	Negative	10-7-20	2	Negative
8	656-20	Acute yellow atrophy of liver	11-22-20	Negative	11-24-20	12	Gas-producing anaerobic bacillus in the blood and kidneys
9	683-20	Septic pneumonia, acute nephritis	12-4-20	Negative	12-9-20	9	<i>Streptococcus</i> in the blood, spleen, peritoneum and pleura
10	691-20	Septicemia	12-4-20	Negative	12-13-20	3	Negative
11	710-20	Radical mastoidectomy	12-18-20	Negative	12-27-20	12	Negative

TABLE 1—Continued  
COMPARISON OF ANTEMORTEM AND POSTMORTEM CULTURES

CASE	NECROPSY NUMBER	POSTMORTEM DIAGNOSIS	DATE OF ANTEMORTEM CULTURE	RESULT OF CULTURE	DATE OF POSTMORTEM CULTURE	LENGTH OF TIME AFTER DEATH, HOURS	RESULT OF CULTURE
12	39-21	Empyema, pyemia	1-24-21	Streptococcus in blood	1-28-21	1	Streptococcus in the blood, spleen, and lungs
13	86-21	Pylonephritis	2-24-21	Negative	2-28-21	13	<i>Bacillus coli</i> in blood and spleen
14	90-21	Vegetative endocarditis, multiple abscesses	2-15-21	Negative	2-28-21	13	<i>Streptococcus hemolyticus</i> in the blood, spleen and abscess
15	105-21	Hypertrophic	3-14-21	Negative	3-16-21	11	Negative
16	111-21	Meningitis	3-17-21	Negative	3-22-21	8	Negative
17	114-21	Nephritis, pulmonary edema	3-23-21	Negative	3-23-21	10	Negative
18	157-21	Hypertrophic	3-26-21	Negative	4-20-21	5.5	Negative
19	187-21	Meningitis	5-7-21	<i>Streptococcus</i> <i>hemolyticus</i> in the spinal fluid	5-7-21	19	<i>Streptococcus hemolyticus</i> in the blood, spleen, brain, kidneys, and lungs
20	216-21	Septicemia, panophthalmitis	5-22-21	<i>Streptococcus</i> <i>hemolyticus</i> in the blood	5-27-21	3	<i>Streptococcus hemolyticus</i> in the blood, spleen, and lungs
21	286-21	Septicemia (abortion)	6-30-21	<i>Streptococcus</i> <i>hemolyticus</i> in the blood	7-4-21	9	Blood negative; <i>Streptococcus</i> <i>hemolyticus</i> in the uterus
22	342-21	Meningitis, otitis media	8-4-21	Pneumococcus in spinal fluid	8-4-21	2	Pneumococcus Type II in the brain, spinal fluid, ears, and splenoid

between antemortem and postmortem cultures to agonal or postmortem invasion. We do not deny the possibility of terminal bacterial infection, but when it occurs it cannot be discarded for it may be the most important contributory factor to the cause of death. This is well illustrated in a patient who came to the Clinic for treatment of abdominal lymphosarcoma (Case 349328). While en route he became acutely ill and soon after reaching the hospital, he died. Necropsy revealed, besides general lymphosarcomatosis of the abdomen, general fibrinous peritonitis and pleuritis; from the peritoneum and pleura as well as from the blood stream a pure culture of *Streptococcus hemolyticus* was isolated. The infection, although terminal, was certainly the immediate cause of death.

The reliability of results may be judged somewhat from examining the type of case in which positive or negative results are obtained. For instance, if patients dying of diseases ordinarily considered noninfectious, such as brain tumor, cerebral hemorrhage, and exophthalmic goiter, give uniformly negative cultures, it would increase our confidence in the results obtained. In our series of 213 cases, forty cases were classified as noninfectious in origin or termination, and it is significant that in these forty cases the results of cultures of various organs and of the blood were negative except in one. Moreover, the time of culturing varied from one to twenty-three hours after death. From this it appears that invasion of the blood stream by the flora of the intact intestinal tract rarely occurs within this time limit.

It may be objected that the cases cited herein lacked an infectious focus from which bacteremia could occur. This point was studied by selecting from our series, cases in which an infectious locus was found. In fifty-one there was a definite locus of infection, such as peritonitis, pleurisy, meningitis, and brain abscess. Since the infection did not appear in these cases in periods of from one to twelve hours, it would seem that so-called postmortem invasion rarely occurs at least within that period of time.

The study of bacteriology postmortem may illuminate, modify, or completely change the cause of death as revealed by clinical and necropsy diagnosis. For example, in a mysterious and apparently unrelated short series of deaths not readily explainable on clinical or pathologic grounds, careful routine bacteriologic studies showed that the deaths probably resulted from contamination of catgut by *Bacillus tetanus*. The importance of these findings is obvious, and in all probability the organism would not have been recovered if the routine mediums employed had not been suitable for the growth of anaerobic bacteria.

In another group of cases the pathologic changes which might be considered a cause of death may be exceedingly meager. In two cases in the series, the sole gross pathologic findings pertaining to the cause of death consisted of a very questionable peritonitis. Culture of the peritoneal fluid and heart's blood yielded *Streptococcus hemolyticus* in pure culture. In the light of such findings the pathologist may explain the cause of death more satisfactorily, especially if death has occurred before frank gross anatomic manifestations appear.



In still another group of cases the bacteriologic findings serve to modify the clinical and pathologic explanation of the cause of death. In one patient in whom the clinical diagnosis of tuberculous meningitis was made, pathologic examination could not exclude the possibility of tuberculosis of the meninges nor could it establish its presence unmistakably. Culture from the brain and spinal cord yielded a green-producing diplococcus which, when injected intravenously in rabbits, exhibited marked localizing power in the central nervous system. Furthermore, sections taken from the patient's brain and stained by the Gram-Weigert method contained gram positive diplococci. A second patient died, who had a long history of Pott's disease and tuberculous otitis media. A clinical diagnosis of tuberculous meningitis was made. This diagnosis was confirmed at necropsy and in addition a culture from the brain yielded a gram-positive diplococcus which also exhibited marked selective affinity for the central nervous system of rabbits. In each of these cases tuberculosis bacilli were found in the spinal fluid previous to death. The superimposed pyogenic infection, while not necessarily the sole cause of death in these cases, was certainly an important contributory cause, a fact difficult or impossible to recognize from the pathologic examination.

The positive and negative blood cultures obtained at succeeding hours postmortem are shown in Fig. 1. The parallelism of the curves is well preserved, except at the thirteenth hour. We conclude, therefore, that there is no sustained progressive increase in positive results at successive hours after death. Postmortem invasion in the time covered by this study does not seem to be of much consequence.

The heart's blood and the spleen were cultured in a series of 189 cases and were found simultaneously positive in sixty-one (36 per cent). The blood alone was positive in eight (4 per cent) and the spleen alone was positive in fourteen (7 per cent). This suggests that the spleen may serve as well as, if not better than, the blood for determining bacteremia, as it is more easily cultured and manipulated.

Of the 213 cases studied, the blood culture was positive in eighty (38 per cent) of the 206 in which blood cultures were made, and the spleen culture was positive in seventy-five (39 per cent) of the 190 cases in which cultures of the latter organ were made. The percentage of positive blood cultures obtained by other workers is as follows: Simmonds, 48 per cent; Fredette, 47 per cent; Gradwohl, 44 per cent; Canavan and Southard, 44 per cent; Otten, 42 per cent; Gay and Southard, 40 per cent; and Richey and Gochring, 29 per cent.

In an analysis of the possible factors contributing to positive blood cultures, it is interesting to note that of twenty-six cases in which the gastrointestinal tract had been injured either by operation or by disease, the blood was positive in 69 per cent. This high incidence of positive cultures may possibly be explained by the fact that in all but one case peritonitis was present. Accordingly, the opportunity for absorption of virulent organisms by the peritoneum was afforded; furthermore, operation and disease processes may decrease the individual's power of resistance and thus aid absorption.

## SUMMARY

1. Bacteriologic cultures were made postmortem in 213 cases.
2. The blood was cultured in 206 cases and it was positive in eighty cases (38.8 per cent).
3. The spleen was cultured in 190 cases and was positive in seventy-five cases (39.4 per cent).
4. The spleen serves as well as, if not better than, the heart's blood for determining a terminal bacteremia.
5. If discrepancies occurred in the results obtained by antemortem and postmortem cultures, these differences were generally explained by the evidence of a superimposed process revealed at necropsy.
6. Uniformly negative results secured in the cases of our series, which are ordinarily regarded as noninfectious in type, strengthens our belief in the reliability of cultures made postmortem.
7. A failure to obtain positive blood cultures in fifty-one cases, in which an abundant focus of infection was demonstrated at necropsy, leads us to believe that invasion of the blood stream after death rarely occurs.
8. Postmortem bacteriology may strengthen, illuminate, or sharply modify the cause of death, as revealed by clinical and necropsy diagnoses.
9. It is possible that terminal invasion may occur, and if it does occur it must not be dismissed, as it may be the most important contributory factor to the cause of death.
10. A sustained, progressive increase in the number of positive results obtained at successive hours after death has not been observed in the period covered by our cases.
11. With strict adherence to a reliable technic, postmortem bacteriologic findings are extremely valuable. We are not making the most of our opportunity at the necropsy table unless routine cultures are made.

# LABORATORY METHODS

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## WIDAL TECHNIC USING STERILIZED CULTURES\*

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BY RUTH GILBERT AND ANNA C. MOORE

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SPECIMENS of blood collected in the Wright capillary tubes for the agglutination test in the diagnosis of typhoid fever have been received at the laboratory in such unsatisfactory condition that for the purposes of a large public health laboratory it has been necessary to require that specimens of dried blood be sent for diagnosis.

Such specimens were tested by a hanging drop Widal technic, using a living 18-hour broth culture of *Bacillus typhosus*. But the results obtained in different laboratories with this method on the same specimens were so discrepant that they could only be accounted for on the supposition that the cultures had been overgrown or were contaminated or were unagglutinable, and the laboratory felt it would be advisable to adopt some other routine procedure.

With a macroscopic technic, killed and standardized suspensions have been found so satisfactory that it seemed desirable, if possible, to use such cultures with a microscopic method.

Widal<sup>1</sup> and Bordet<sup>2</sup> as early as 1896 called attention to the fact that a culture could be killed and remain agglutinable. In the next year Wright and Semple<sup>3</sup> reported favorably on the use of cultures killed by heat for agglutination tests, and Foerster<sup>4</sup> and Widal and Sieard<sup>5</sup> used cultures killed with formalin instead of heat and found that they were no less agglutinable and possessed better keeping qualities. Fieker<sup>6</sup> in 1903 prepared his "typhus-diagnosticum," consisting apparently of a typhoid culture killed in some manner. The actual preparation is not described and seems to have been a trade secret. Lion,<sup>7</sup> Gramann<sup>8</sup> and Meyer<sup>9</sup> have reported favorably on the use of this diagnosticum. Ruediger<sup>10</sup> in 1904 carried on macroscopic agglutination tests with a culture killed with 1 per cent formalin but not standardized.

Dreyer in 1906 introduced his "standard" method of macroscopic agglutination with killed cultures. His original article was translated for English readers in 1909. His method in brief is as follows: A culture of *Bacillus typhosus* is grown in veal peptone bouillon for 24 hours at 37° C. and then killed by the addition of 0.1 per cent of the ordinary 40 per cent solution of formalin. When subcultures show it sterile, it is standardized as to density and agglutinability. A macroscopic tube agglutination is employed for the test of patients' serum.

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Received for publication Aug. 27, 1921.

This method with some slight modifications has been used and favorably reported upon by Murray,<sup>12</sup> Davison,<sup>13</sup> Walker,<sup>14</sup> Glynn,<sup>15</sup> Donaldson and Clark,<sup>16</sup> Krumbhaar and Smith<sup>17</sup> and Fennel.<sup>18</sup> Priestly<sup>19</sup> thinks the technic too complicated. Morse<sup>20</sup> found suspensions in salt solution from agar slants made up to a standard opacity more satisfactory than broth cultures prepared by Dreyer's method.

Using killed and standardized cultures, various simple macroscopic slide and tube agglutination tests have been devised, which while diagnostic do not give accurate quantitative results, as only one dilution of patients' serum is used in the test. Among these may be mentioned the tests of Bass,<sup>21</sup> Bass and Watkins,<sup>22</sup> Johns,<sup>23</sup> Gillman<sup>24</sup> and Coles.<sup>25</sup>

Since the advantages of sterilized cultures for macroscopic agglutination tests have been so generally demonstrated, it seemed desirable, if possible, to use a killed culture with a simple microscopic Widal technic. Two cultures were accordingly prepared, one a broth culture of *B. typhosus* prepared according to Dreyer's method and another an emulsion from agar slants of *B. typhosus* washed off in 0.5 per cent salt solution and killed with 0.1 per cent of commercial formalin. These cultures were well shaken and put in the ice box and subcultured daily. At the end of three days they were found to be absolutely sterile. A bacterial count was then made on each culture.

On the basis of the count, ten dilutions of each culture were made varying from 500 million to 5000 million per c.c. Each of these dilutions was tested by the hanging drop method against several dilutions of typhoid immune serum, normal human serum diluted 1-20, and 0.5 per cent salt solution, to determine the lowest dilution of culture which would give definite agglutination with typhoid immune serum and no appearance of agglutination with normal human serum or 0.5 per cent salt solution. On the basis of these tests the 3000 million concentration was adopted as the standard. This was later abandoned in favor of the 1000 million concentration as the 3000 million was found so heavy as to be confusing in negative readings.

Using blood specimens previously found to contain typhoid agglutinins as well as those previously found to contain no typhoid agglutinins hanging drop tests were made to determine the optimum period of incubation for use with the killed cultures. Incubation for one hour, one and one-half hours, and two hours at 37° C. showed no appreciable difference in the degree of agglutination so one hour was chosen as the standard incubation period for use with the killed cultures, the same length of time which had been found suitable for the Widal tests with a living culture.

A series of forty comparative tests was made by our routine Widal technic using the living 18-hour broth culture and the two killed cultures with very discouraging results. Of those 40 tests, 13 or 32.5 per cent gave a positive agglutination with the living culture. Of those that gave positive agglutination with the living culture 61.5 per cent were positive with the killed agar slant emulsion and 23.1 per cent were positive with the killed broth culture. Even in the cases where the killed cultures gave agglutination, it was in no instance as strong as that given with the living culture.



Seven months later another broth culture and another agar slant emulsion of the Bender\* strain of typhoid were prepared according to the methods previously described. These cultures were killed as before with 0.1 per cent of commercial formalin and counted, four different dilutions being counted and an average taken to insure as accurate a count as possible.

Using a 1000 million concentration of the two killed cultures and an 18 hour living culture with our routine Widal technic, a series of parallel tests was run on 237 specimens of blood sent in for the Widal test. The results

TABLE I

COMPARISON OF RESULTS OBTAINED IN FORTY MICROSCOPIC WIDAL TESTS USING A LIVING BROTH CULTURE AND THE FIRST SET OF KILLED CULTURES.

Living Culture	POSITIVE AGGLUTINATION			PARTIAL AGGLUTINATION			NO AGGLUTINATION		
	13, 32.5 PER CENT			8, 20 PER CENT			19, 47.5 PER CENT		
	Positive No. %	Partial No. %	Negative No. %	Positive No. %	Partial No. %	Negative No. %	Positive No. %	Partial No. %	Negative No. %
Killed Cultures									
Killed Broth Culture	3 23.1	9 69.2	1 7.7	0 0.0	3 37.5	5 62.5	0 0.0	1 5.3	18 94.7
Killed Agar Slant Emulsion	8 61.5	2 15.4	3 23.1	0 0.0	5 62.5	3 37.5	0 0.0	1 5.3	18 94.7

TABLE II

COMPARISON OF RESULTS OBTAINED IN 237 MICROSCOPIC WIDAL TESTS USING A LIVING BROTH CULTURE AND THE SECOND SET OF KILLED CULTURES.

Living Culture	POSITIVE AGGLUTINATION			PARTIAL AGGLUTINATION			NO AGGLUTINATION		
	63, 26.6 PER CENT			58, 24.5 PER CENT			116, 48.9 PER CENT		
	Positive No. %	Partial No. %	Negative No. %	Positive No. %	Partial No. %	Negative No. %	Positive No. %	Partial No. %	Negative No. %
Killed Cultures									
Killed Broth Culture	3 4.8	44 69.8	16 25.4	0 0.0	15 25.9	43 74.1	0 0.0	0 0.0	116 100.0
Killed Agar Slant Emulsion	14 22.2	42 66.7	7 1.1	0 0.0	21 36.2	37 63.8	0 0.0	0 0.0	116 100.0

obtained in this second series of tests were even more unsatisfactory than the results obtained with the first set of killed cultures. Of the 237 specimens tested 63 or 26.6 per cent gave a positive agglutination with the living culture. Of those that were positive with the living culture, 22.2 per cent were positive with the killed agar slant emulsion and 4.8 per cent were positive with the killed broth culture. In no instance were positive results obtained with the use of killed cultures with specimens found negative when tested with living cultures. The fact that the second set of killed cultures compares even less favorably than the first set with the results obtained with the living culture, may be accounted for by the fact that the tests were made on the

\*A strain of the typhoid bacillus obtained originally from the Bender laboratory but under cultivation for six years on media before it was used in these tests.

second set of cultures over a much longer period of time, a large number of the tests having been made when the cultures were more than six months old.

As the impracticability of a microscopic Widal technic using killed cultures seemed definitely established by these two sets of unsatisfactory results, macroscopic tube agglutinations were then run to determine whether the killed cultures would prove satisfactory for use in a macroscopic test. Different concentrations of the killed cultures were tested and the 3000 million concentration was found to give the most definite agglutination in macroscopic tests. Incubation for two hours in dry air at 37° C., in the water-bath at 37° C. and in the water-bath at 55° C. gave an almost identical degree of agglutination though the readings were most clear cut when the tests were incubated in the water-bath for two hours at 55° C.

Using a living 2-hour broth culture and the 3000 million concentration of the two killed cultures the serums of a number of typhoid convalescents and of persons who had shown a positive Widal after inoculations with typhoid vaccine, were tested. Both the living and the killed cultures were agglutinated by these serums in the final dilutions of 1-50 and 1-100. The agglutination obtained with the killed agar slant emulsion was the most definite and clear cut. These macroscopic tests were done when the killed cultures were almost a year old and indicate clearly that such cultures may be used with reliable results over long periods of time in a macroscopic test.

#### SUMMARY AND CONCLUSIONS

Killed cultures of *Bacillus typhosus* have proved unsatisfactory for the microscopic agglutination test for typhoid. They are satisfactory, however, for use in macroscopic agglutination tests.

If more comparable results are to be obtained in different laboratories, it may be desirable to have carefully standardized killed cultures prepared at a central laboratory for distribution. If this were done, it would be necessary to adopt the macroscopic technic for performing the test.

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## SUGGESTIONS FOR THE DETERMINATION OF URIC ACID IN BLOOD\*

BY L. BAUMAN, M.D., AND L. M. KEELER, A.B., NEW YORK CITY

WE have replaced the standard uric acid solution with Lovibond tintometer glasses. The Folin-Wu<sup>1</sup> method is followed, using 20 c.c. of protein-free filtrate corresponding to 2 c.c. of blood. The blue compound is developed in 25 c.c. flasks without the use of sodium sulphite as the glasses are calibrated against the standard uric acid solution of Benedict and Hitchcock.<sup>2</sup> The glasses are placed over the upper end of the immersion cylinders, or prisms, of the Duboseq colorimeter. A red glass (0.4) is placed over the unknown solution and a blue glass (2.9) over the opposite prism, the latter being immersed to the 20 mm. mark in distilled water. It requires a depth of 17.6 mm. of the blue solution obtained with 0.1 mg. of uric acid diluted to 50 c.c. to match the colored glasses. The following formula is used to obtain the amount of uric acid (in milligrams) contained in 100 c.c. of blood.

$$\frac{\frac{17.6 \times 100}{\text{Reading of unknown}}}{2 \times 2}$$

The glasses may also be used with the Bock-Benedict colorimeter if the red glass is placed over the immersion cylinder and the blue in front of and parallel to the standard cell.

We have found the glasses to be satisfactory for the estimation of uric

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acid in practically all samples of blood which are ordinarily sent to a hospital laboratory.

The advantages of the glasses are, first, that they shorten the time of the procedure and second, that they remove the uncertainty arising from the possible decomposition of the standard uric acid solution. The annoying precipitation occasionally encountered in this method may be avoided by the addition of 3 drops of half saturated (in the cold) gum acacia solution. A crystal of thymol is added to the gum solution to avoid bacterial decomposition. This device has enabled us to carry through about 300 determinations without a single precipitation. The acacia probably acts as a protective colloid.

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### THE USE OF OPEN DELIVERY TUBES IN THE DISTILLATIONS WHEN DETERMINING UREA AND NONPROTEIN NITROGEN IN BLOOD\*

BY GUY E. YOUNGBURG, PH.D., N. Y.

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IN the distillation of the ammonia formed, either in the urea determination or in the determination of nonprotein nitrogen after micro-Kjeldahl digestion, it is not necessary that the delivery tube shall dip into the acid solution in the receiver, provided the latter be kept at or below room temperature. This is easily accomplished by placing the receiver in a beaker of water. The condensation of the water vapor is complete and since only small quantities of ammonia (maximum about 1.75 mg. N) are involved there is no loss. Results obtained are shown in Tables I and II.

The procedure followed was that of Folin and Wu in their System of Blood Analysis,<sup>1</sup> except the following: In the open method the delivery tube reached only near (1 to 2 cm. above) the surface of the acid solution in the receiving tube (200 × 20 mm.) which in turn was placed in a 600 c.c. beaker nearly full of tap water. The water submerges the tube about  $\frac{2}{3}$  of its length. Since the tube was not to be slipped off from its rubber stopper, which contains a slit, the total distillation period was, as prescribed by Folin and Wu, five minutes. While a shorter and more vigorous distillation period leads to no loss of nitrogen, yet it is not preferable from other standpoints. The same beaker of water can be used for several determinations but after that the water must be renewed.

A Kober colorimeter was used and the readings are an average of three. The results of the open tube method are on the average practically iden-

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\*From the Laboratory of Biological Chemistry, Medical Department, University of Buffalo, Buffalo. Received for publication, September 15, 1921.



TABLE I  
NITROGEN RECOVERY IN MICRODISTILLATION OF AMMONIA USING OPEN AND CLOSED  
RECEIVERS\*

DETERMINA- TION NO.	BY OPEN DISTILLATION			BY CLOSED DISTILLATION		
	COLORIMETER READING	NITROGEN RECOVERED		COLORIMETER READING	NITROGEN RECOVERED	
	mm.	mg.	per cent	mm.	mg.	per cent
1	19.7	0.0765	102	20.4	0.0735	98
2	20.5	0.0735	98	19.8	0.0757	101
3	20.9	0.0712	95	20.0	0.0750	100
4	20.6	0.0727	97	21.7	0.0690	92
5	21.0	0.0712	95	20.3	0.0735	98
6	20.8	0.0720	96	20.6	0.0727	97
7	20.1	0.0742	99	20.0	0.0750	100
8	20.6	0.0727	97	20.3	0.0735	98
9	19.5	0.0772	103	19.7	0.0765	102
10	20.6	0.0727	97	19.6	0.0765	102
Av.	20.4	0.0735	98	20.2	0.0742	99

\*The determinations were made upon 5 c.c. portions of a standard ammonium sulfate solution (5 c.c. = 0.075 mg. N), in the same way as if blood filtrates.

TABLE II  
DETERMINATION OF UREA NITROGEN IN BLOOD, USING OPEN AND CLOSED RECEIVERS IN THE  
DISTILLATION OF THE AMMONIA FORMED

BLOOD	DETERMINA- TION NO.	BY OPEN DISTILLATION		BY CLOSED DISTILLATION	
		COLORIMETER READING	NITROGEN FOUND	COLORIMETER READING	NITROGEN FOUND
		mm.	mg. per 100 c.c.	mm.	mg. per 100 c.c.
Beef	1	21.6	13.9	21.8	13.7
	2	21.1	14.2	20.6	14.5
	3	20.1	14.9	21.1	14.2
	4	21.3	14.1	21.3	14.1
	5	21.2	14.1	20.4	14.7
	Av.	21.1	14.2	21.0	14.3
Sheep (Sample A)	1	24.2*	24.7	24.7*	24.3
Sheep (Sample A) with added urea (300 mg. per 100 c.c.)	1	18.4*	326	18.1*	331
	2	17.9	336	19.1	314
	3	17.9	336	18.9	318
	4	18.2	330	17.7	339
	5	18.1	331	17.6	342
	6	18.2	330	17.9	337
	7	18.2	330	18.6	323
	8	18.0	333	19.2	313
	9	18.2	330	18.2	330
	10	18.8	319	18.9	318
	Av.	18.2	330	18.4	326

\*An aliquot part of distillate taken for Nesslerization in this series.

tical with those of the closed tube method and the individual determinations agree as closely as is to be expected for colorimetric work. Where urea was added in order to give the maximum amount which has been found in pathologic blood,<sup>2</sup> there was no loss of nitrogen.

While no check determinations were made for the estimation of non-protein N by distillation with open tube instead of by aeration,<sup>3</sup> the process of distillation is the same as for urea and further experiments were considered superfluous.

## ADVANTAGE OF OPEN TUBE DISTILLATION

Kjeldahl distillations in general are known to offer the difficulty that the receiver contents are often sucked back into the distilling liquid. In the microdeterminations it is extremely annoying and many determinations are lost in this way even though the technician be one of the best. Open tube distillation overcomes the difficulty. The same feature, however, cannot be applied to the macro-Kjeldahl distillation because too large amounts of ammonia are involved and more or less nitrogen is lost.

The writer has found that ordinary paraffin oil should not be used in preventing foaming during distillation. While a good grade of oil which was only slightly colored was at first employed, a disturbing effect was noted when attempting to make the final color comparison and when noting the nitrogen recovery. A slightly different color effect can usually be observed when comparing with the standard. This is due to some small part of the oil which distills over, the particles of which cause a slight turbidity which makes

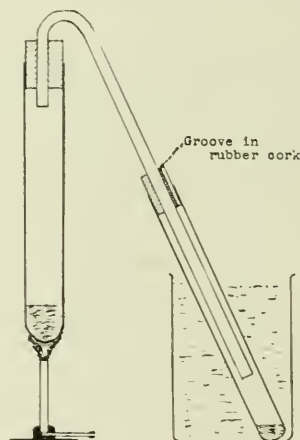


Fig. 1.—Open-tube distillation apparatus.

the color comparison difficult and gives results which are a little low. When this effect was noted an especially purified paraffin oil, such as is sold for internal use, was employed. Such oil is perfectly colorless and water clear, and none distills over. The particular brand employed was the "American Oil" of Parke, Davis & Co.

The perforated distillation tube of Watson and White,<sup>4</sup> as a means to prevent foaming, was tried with the hope to omit the paraffin oil. While preventing foaming to some extent such a tube is not efficient enough to answer the purpose.

Sheep's blood\* was used in the experiments reported because from the standpoint of foaming it has given the writer more difficulty than other normal bloods and should thus be a more severe test on the methods employed.

\*From five samples of fresh sheep's blood collected at the slaughterhouse, the urea N found was 18.5 to 24.7 mg. per 100 c.c., the average being over 20 mg. This is considerably higher than that of human or beef bloods.

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## A SIMPLE METHOD OF COUNTING THE CELLS IN CEREBROSPINAL FLUID\*

By CHAS. F. CARTER, B.S., M.D., DALLAS, TEXAS

WE have used the following method of counting the cells in cerebrospinal fluid for about two years and during this time it has given entire satisfaction. We selected it because of its simplicity and because the counting chamber used was the Levy counting chamber with double Neubauer rulings, this being the one in almost universal use for blood counting. The diluting fluid is that recommended by Levinson† (Methyl violet 0.2 gm., glacial acetic acid 5 c.c. and water to make 100 c.c.). With a pipette (1 c.c.) accurately mix equal parts of cerebrospinal fluid and diluting fluid (0.5 c.c. of each). Adjust cover of counting chamber and with a fine capillary pipette, allow preparation to flow over both rulings, using the same precautions as in making a preparation for a blood count. Let settle five minutes for the cells to stain and for the preparation to become even. Count all the cells in the four corner blocks of sixteen large squares used for counting white blood cells and the central block of four hundred small squares used for the red blood count. Move to the other ruling and repeat the process. The total number of cells counted multiplied by two gives the total cells per cubic millimeter. We use the high dry lens because the cells are not mistaken for debris and the differential count may be performed at the same time.

## WHAT ARE THE ATMOSPHERIC MOISTURE REQUIREMENTS OF BACTERIA‡

By NICHOLAS KOPELOFF, PH.D., AND STERNE MORSE, M.D., NEW YORK CITY

EVERYONE who has occasion to grow bacteria is familiar with the necessity of keeping the incubator properly supplied with moisture. A thorough search of the literature, however, reveals a surprising absence of investigations concerning the atmospheric moisture requirements of bacteria.

\*Received for publication, October 15, 1921.

†Levinson, Cerebrospinal Fluid, C. V. Mosby Co.

‡From the Research Laboratories of The New York State Psychiatric Institute, Ward's Island, N. Y.

Received for publication, October 15, 1921.

The majority of text books on bacteriology contain statements to the effect that all bacteria require a certain amount of moisture for their development and that the destructive action of drying affects various species differently. Beyond the abortive experiments of Wolf<sup>1</sup> and Weigert,<sup>2</sup> it has been difficult to find any rigidly controlled data which established the minimum, optimum and maximum moisture requirements of this group of microorganisms.

Obviously the importance of such information would vary with the problem under consideration. In order to achieve uniform cultural conditions, we were led to devise an incubator which would control the relative humidity of the atmosphere as well as temperature. To operate it to the best advantage it was first necessary to establish the atmospheric moisture requirements of bacteria. This paper may be regarded as a preliminary note of these investigations now in progress.

It would be superfluous to describe in detail the various methods devised and discarded in an attempt to control the moisture requirements of bacteria. Suffice it to say that simplicity, economy and efficiency have been achieved by the use of sterile Petri dishes. Instead of the usual arrangement of placing a top and bottom together, two tops or two bottoms are joined and sealed with adhesive tape ( $\frac{1}{4}$  inch) around the equator. Agar to support the growing culture is placed in the upper dish and solutions of dehydrating agents of varying concentrations, yielding a definite vapor tension and relative humidity are placed in the lower dish. The procedure is as follows: A layer of glucose agar 2 mm. thick (15 c.c.) is poured into one dish of each pair. It is our custom to add about .002 gm. of Brom Cresol Purple to every 100 c.c. of agar as an indicator for the development of acidity. On the hardened agar surface is deposited 0.1 c.c. of a suspension of the proper dilution (previously determined), of the organism used. This yields only a surface growth which is essential since variations of growth in the depth of the media are more difficult to measure and control. In order to obtain a uniform distribution of colonies on the plate a bent glass tube is stroked gently over the surface. The plate is then inverted and 35 c.c. of solution of known concentration glycerine, calcium chloride or sulphuric acid are added to the other dish. The two dishes are tightly bound together with adhesive tape and incubated at 37.5° C. for 18 hours.

By means of a Leitz projection photographic apparatus it has been possible to obtain a permanent magnified record of the growth under varying conditions of moisture. Furthermore, accurate measurement of the individual colonies is a part of the routine examination, which is carried out very easily and rapidly.

Some suggestive results have been obtained with *B. Coli*, *Staph. aureus* and *B. subtilis*. Saturated calcium chloride (B.P. 132° C.) with and without an equal volume of water were compared with water as a control; likewise glycerine (sp. gr. 1.258 at 20° C. B.P. 157° C. under a pressure of 205 m.m.) in the same proportions.

In Fig. 1, it will readily be seen that the maximum growth is obtained in



a humid atmosphere (i.e. where water is present). The minimum growth appears in the least humid atmosphere (i.e., where the concentrated calcium chloride, yielding a relative humidity of approximately 35-48 per cent, and glycerine, yielding a relative humidity of 0-55 per cent, acted as strong dehydrating agents). The solutions of half strength occupy an intermediate position, (yielding relative humidities of approximately 75-85 per cent). It will, of course, be noted that these experiments were not conducted with constant water vapor tensions under the conditions described, for the reason that there occurs a progressive dehydration of the medium and dilution of the dehydrant. This is obviated in an apparatus now being used and which is an adaptation of one described by Orme and Masson and Richards<sup>3</sup> in investigations on the adsorption of water by cotton fiber.

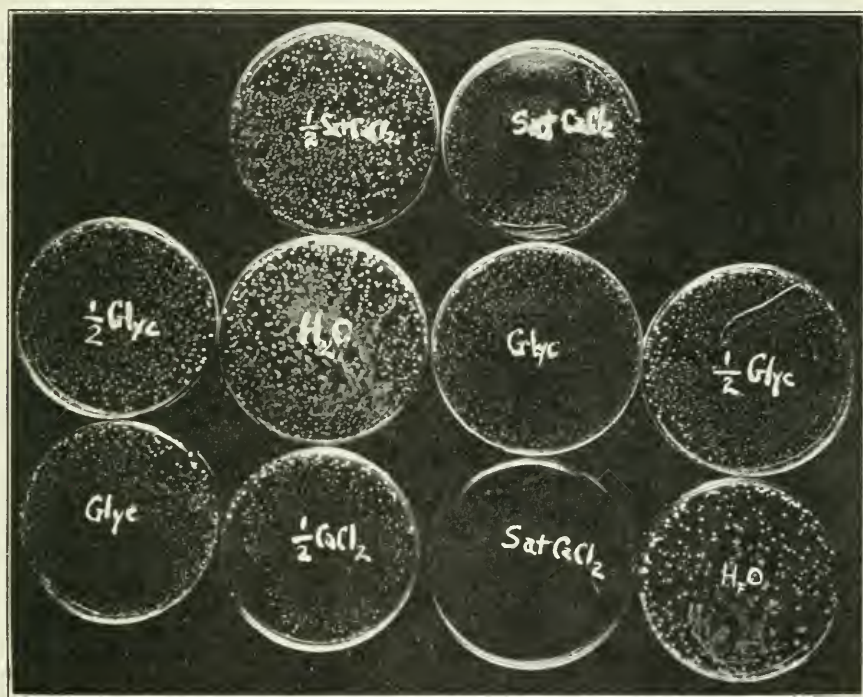


Fig. 1.—Plates with *B. Coli* under varying atmospheric moisture conditions.

Duplicate plates as follows:

H<sub>2</sub>O—water; Sat. CaCl<sub>2</sub>—saturated calcium chloride;  $\frac{1}{2}$  Sat. CaCl<sub>2</sub>—one-half saturated calcium chloride; Glyc.—concentrated glycerine;  $\frac{1}{2}$  Glyc.—one-half concentrated glycerine.

In the experiment described it was apparent that the media was subjected to a very marked drying out where the atmosphere was least humid (concentrated calcium chloride and glycerine) and somewhat less dry where half concentrations were employed. The application of this phenomenon to the drying out of culture media in test tubes and Petri dishes is obvious. By maintaining an incubator at a relative humidity of 10 to 40 per cent (at 37.5° C.) it was found that the above results obtained with solutions were practically duplicated. This means that it is essential to keep sufficient

moisture in the atmosphere of the ordinary bacteriologic incubator in order to develop characteristic colony formation.

It is especially interesting in this connection that striking differences in colony formation were noted. Where there was sufficient moisture in the atmosphere, *B. coli* gave its usual characteristic growth. (Fig. 2.) However on the plates where a dry atmosphere obtained, the colonies were much smaller, with very irregular outline and surface. (Figs. 3 and 4.)

It is our purpose to define more closely the limits for the variations in

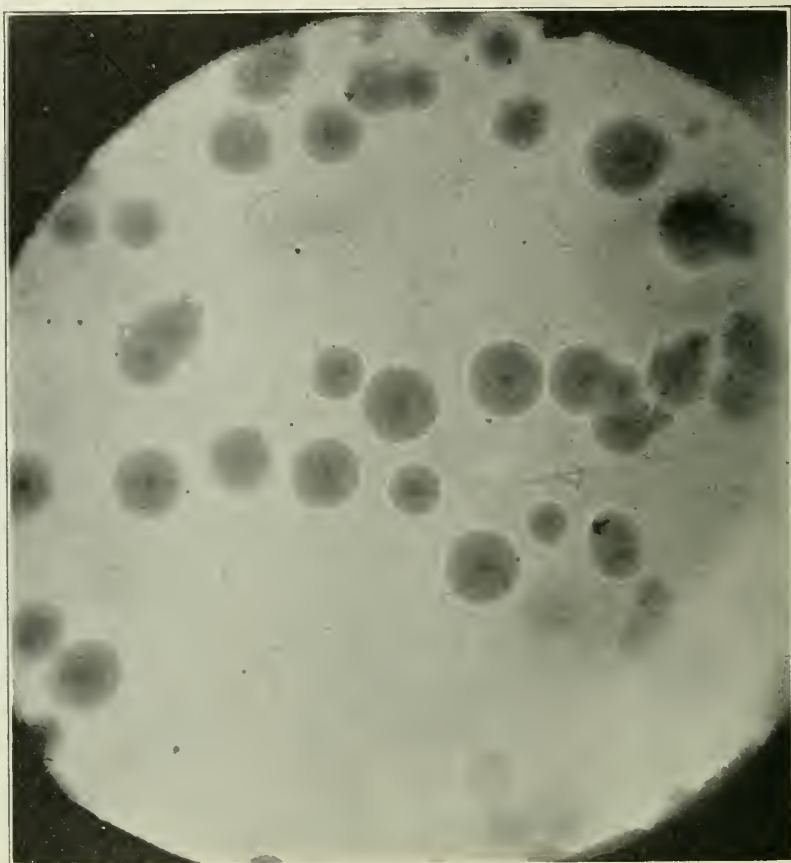


Fig. 2.—*B. Coli* in humid atmosphere (water) magnified  $8\frac{1}{2}$  diameters.

growth of a large number of bacteria when subjected to varying conditions of atmospheric moisture and more generally to arrive at the numerical relations of the rates of growth of various microorganisms under varying water vapor tensions.

It is also possible to prepare solutions which have fixed tensions of various gases such as carbon dioxide, ammonia, hydrogen sulphide, methylamin and the like to which cultures may be subjected in a similar manner. Such experiments are beyond the scope of the present paper and consequently will be reserved for future publication.

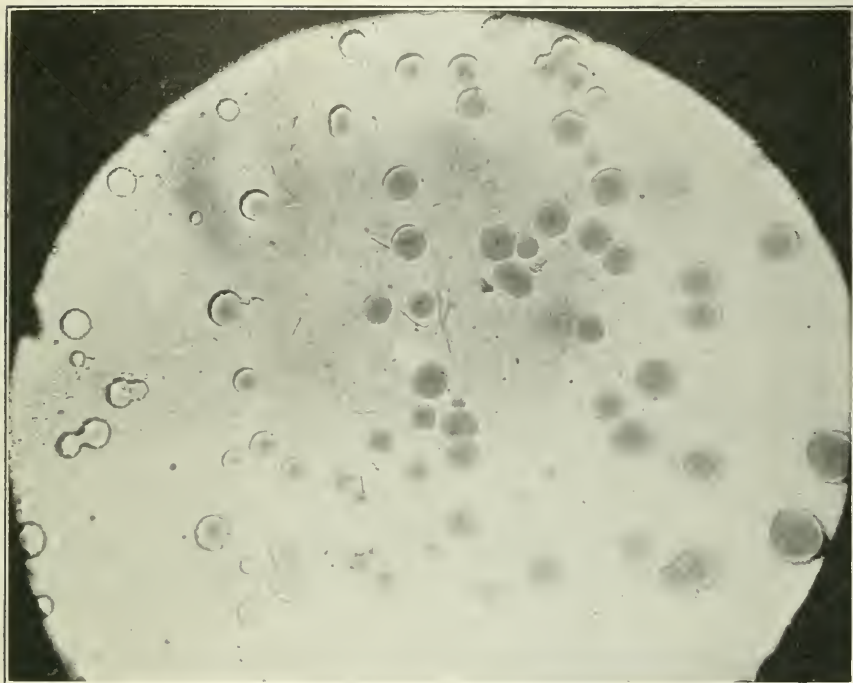


Fig. 3.—*B. Coli* (inoculation identical with Fig. 2) in partially dry atmosphere (one-half saturated calcium chloride) magnified  $8\frac{1}{2}$  diameters.



Fig. 4.—*B. Coli* (inoculation identical with Figs. 2 and 3) in dry atmosphere (saturated calcium chloride) magnified  $8\frac{1}{2}$  diameters.



## SUMMARY

1. Methods have been devised for controlling the atmospheric moisture conditions in the growth of bacteria.

2. The results thus obtained indicate that bacteria have definite variations in rate of multiplication and amount of growth under changing conditions of moisture.

3. In order to obtain characteristic colony formation and prevent the drying out of media, it is essential that an adequate supply of moisture be present in the bacteriologic incubator.

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## AN ADAPTED MASK FOR BASAL METABOLISM APPARATUS\*

BY PHILIP B. NEWCOMB, B.S., M.D., LOS ANGELES, CALIF.

EXPERIENCE in the use of apparatus of the closed circuit type for basal metabolic determination has proved to many observers that the usual mouth piece with nose clamp is the most frequent source of error in results and of discomfort and apprehension to the patient.

It is scarcely to be denied that the comfort and cooperation of the patient are very potent factors in the correct and successful application of this procedure. More especially is this seen to be true when it is considered that the individuals in whom the knowledge of the basal metabolic rate is most valuable are generally of the neurotic type. With even the best of volitional effort upon the part of the patient, false readings are liable to occur from irregular or forced respiration, salivation with consequent choking sensation and swallowing of oxygen, together with leakage from the angles of the mouth. All these elements of potential inaccuracy are intensified whenever the person undergoing the test becomes frightened, acutely discommoded or subject to actual pain by the use of the irksome mouth piece and nasal clamp. Masks of various types have been used in the effort to obviate such difficulties and uncertainties, but some have been open to charge of error from leakage, while others are cumbersome and expensive.

It is the object of this communication to propose the use of a simple, easily procurable mask and to outline a ready method of its adaptation to the Benedict apparatus. This method has, in the writer's experience, eliminated many of the difficulties mentioned, places no tax upon the respiration

\*From the Pacific Wassermann Laboratories, Los Angeles, Calif.  
Received for publication, October 24, 1921.



and has proved satisfactory in respect to retention of the closed circuit of air and oxygen.

The mask itself is of a standard type in use for the administration of nitrous oxide anesthesia, having a pneumatic rim around the lower border, which it is usually found preferable to inflate.

As obtained from the dealer, the acute angle of the egg shaped base of the mask points in a direction away from the inlet tube (*A*) and, if the Benedict apparatus is used from the side or below the patient's head, as is customarily the case, the position of this angle should be reversed. Such correction can be accomplished by a forcible twist between the celluloid (*E*) and metal (*A*) portions so that, when the mask is adjusted to the face, the smaller angle will rest over the nose and the broader portion upon the lower jaw or chin. There is an exhalation valve of the flutter type at (*F*), which is closed off by firmly screwing down the milled head at (*B*), making this portion air tight. The inlet tube (*A*) of the mask is connected with the distal arm of the respiration apparatus (*D*) by means of a piece of strong rubber tubing or hose (*C*) about two inches in length, having an inside diameter of seven-eighths inch. If desired, the connection can even be made through the

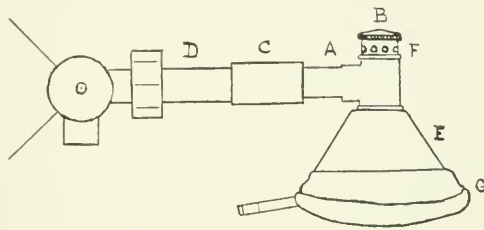


Fig. 1.—*A*, inlet tube of mask. *B*, milled head cut out. *C*, rubber tube connection. *D*, metal tube of respiration apparatus. *E*, celluloid body of mask. *F*, exhalation valve. *G*, pneumatic rim of mask.

agency of the original rubber mouthpiece itself, acting as a tube. This flexible connection (*C*) permits freedom of motion in adjusting the mask, a mobility not attainable when the rigid arm alone is employed. When ready to perform the test, the mask is applied over the nose and mouth and secured by tapes. For this latter purpose, it has been found convenient to use a towel or napkin, six to eight inches wide and twelve inches in length, to each long or vertical edge of which are attached ten linen tapes about eighteen inches in length, or a "many-tailed" bandage can be fashioned of similar size from strong muslin and serve a like purpose. The patient's occiput rests upon this towel or bandage placed over the pillow and the tapes are tied to those of the opposite side across the mask. A little ingenuity in adjustment is required and it is often found preferable to unite several pairs of tapes to those diagonally opposite (i.e., upper right hand corner to lower left, etc.), in a sort of "basket weave" and to include the central metallic portion of the mask with a twist of each tape for added security. The arrangement is completed with horizontal union of two pairs of tapes directly over the lower and upper extremities of the mask. Leakage, if it should occur, is apt to

be from the top portion of the mask at the nasal notch, which contingency can, however, be guarded against through the exercise of firm but gentle pressure by the assistant upon the mask in this locality.

For routine work, the device and method outlined have proved of distinct advantage and it has been possible thereby to obtain corrected readings in instances where former results with the mouthpiece and nasal clip were obviously not in accord with clinical manifestations, and to overcome strenuous objection in persons timorous either by nature or as an outcome of previous unpleasant experience with the original breathing appliance. The mask and connections are readily amenable to thorough cleansing with soap and water and disinfection by germicidal solutions.

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### A PLEA FOR THE STANDARDIZATION OF THE TRAINING FOR LABORATORY TECHNICIANS\*

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BY GARNET B. GRANT, B.S., M.D., AND ERIC R. WILSON, M.S., M.D.,  
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THE article by Dr. Gradwohl, appearing in the August issue of the *Journal of Laboratory and Clinical Medicine*, should find ardent support from all those who are interested in this work and particularly those medical men operating the so-called commercial laboratories.

We have long hoped that a move would be set on foot for standardizing the training of technical laboratory help, this move fostered by the Educational Council of the Am. Med. Assn., and in turn taken up by the various state boards, so that laboratory help would come under the jurisdiction of the state, very much in the same way as nurses do now.

The lack of proper training for this branch of work is in our experience somewhat appalling. The average so-called technician is, to say the least, poorly trained. This last remark we would like to qualify by further stating that while some may be very well trained in a few things, and have a good knowledge of ordinary routine work, beyond this point the average technician is hopelessly lost. A few have a good fundamental training and a smattering of how to solve the many difficult problems encountered in the so-called commercial laboratory. An occasional one, and these are rare, is able to take care of all work and is properly trained in all the branches that are included in the technician's field.

It has been our sad experience in the past year to have temporarily employed, at least, thirty technicians, a good many of them having had of the following qualifications: Three to five years of actual laboratory experience. This included bacteriology, serology, blood chemistry, etc. Others had been bacteriologists for small towns in the East and Middle West. Others had

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\*Received for publication, September 19, 1921.

M.D. degrees, others B. S. degrees and unfortunately not two per cent of this number could properly take care of the work they claimed to be efficient in.

The following question has been asked of fifty applicants who have applied to us for positions, claiming good working knowledge of bacteriology, and in no case has it been properly answered.

"How do you differentiate streptococcus from pneumococcus?" The answers were numerous and varied, but none were answered correctly.

Very few, if any, had experience in the use of the Polariscope, none had ever heard of a polarizing microscope used in the identification of crystals. A few were able to do a little blood chemistry, but none seemed to understand the underlying principles of chemical analyses. It is only the occasional one that is able to correctly make up a normal solution.

The rapid advance of laboratory methods and use of the medical laboratory in making or aiding in the diagnosis of disease, as well as the important part it plays in preventive medicine and hygiene, make it necessary to standardize the training of the laboratory workers.

We are firmly under the impression that the medical laboratory comes strictly within the domain of medicine, or in other words, we consider this as far as the directors of laboratories are concerned, and just as we believe that the time is close at hand when the so-called lay laboratory will be a thing of the past, supplanted by laboratories, the work of which will be supervised by doctors of medicine, who in turn are licentiates to practice medicine and surgery in those states in which their laboratories are operated, so in turn do we believe that the actual technical help in such laboratories should have the proper training and be required to pass a state board examination and become registered in the state in which they work.

We fail to see how the proper training of such help can be accomplished in less than two years. They should have a good working knowledge of organic and inorganic, as well as, physiological chemistry. They should be competent bacteriologists, having a good knowledge of protozoology, as well as, parasitology, be thoroughly familiar with all routine work, urinalysis, blood counts, etc., as well as being familiar with tests that are frequently used, but not considered as routine. They should have at least three months' experience in serology and thoroughly familiar with the theory of complement-fixation tests, and last, but not least, they should be familiar with basal metabolism, as well as blood chemistry.

Such a course, would eliminate those of weaker mentality, as well as keep out those who believe that three to six months' training is sufficient to turn out good technicians, in other words it would create a profession for the laboratory worker, as well as doing much towards standardizing the work.

## THE MAKING OF COLLODIUM SACS\*

BY NATHAN MUSKIN, M.D., AND LOUIS SIEGEL, NEW YORK, N. Y.

THE greatest difficulty in the making of dialysis membranes is the separation of the collodium sac from its glass mold. To obviate this difficulty the following technic has been successfully used: Place a small collar of paper one inch wide in the mouth of the tube before adding the collodium (see cut). Then pour the collodium into the tube filling it completely. The collodium is then poured back into stock and the tube allowed to dry by hanging upside down for a few minutes. The tube is then placed in a 55° C. thermostat (drying oven) for one-half hour, or allowed to stand at room temperature for

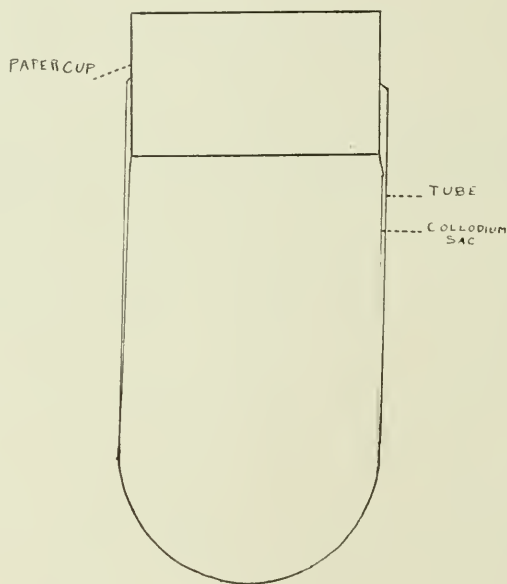


Fig. 1.

several hours, to allow the collodium to dry. At the end of this time the collar is gently pushed in at some point and distilled water poured in between the collodium sac and the glass container. As soon as the container is full of water the sac can be easily removed. By this method as many as twelve bags can be loosened in ten minutes. The paper collar is a very convenient handle for the sac, both in the separation from the glass mold and afterward.

\*From the Pathological Laboratory of the Mount Sinai Hospital.  
Received for publication, September 12, 1922.



# *The Journal of Laboratory and Clinical Medicine*

VOL. VII.

JUNE, 1922

No. 9

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Washington, D. C.

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## *EDITORIALS*

### *Liver Function*

THE liver possesses a large number and considerable variety of functions. No other organ probably plays so important a rôle in the metabolism of such diverse substances as does the liver. Liver cells remove materials from the circulating blood, and after transforming them chemically and biologically, either return the altered products immediately to the blood, or excrete them with the bile, or store them for a longer or shorter period of time. These cells secrete bile, form and hydrolyze glycogen, store fat, form urea from ammonia, serve as a temporary storehouse for iron, produce fibrogen, and detoxicate what poisonous substances customarily find their way from the intestines into the portal circulation. These functions we already know. Presumably there are more. Dissociated jaundice in which bile pigment is shunted into the blood while bile salts pass, as normally, into the intestines is an excellent example of the very delicate functional adjustment of the liver cells.

There is some evidence suggesting considerable tendency on the part of damaged hepatic parenchyma to return to normal. Apparently the cells may functionate well in some of their tasks while being unable to success-

fully carry out others. It is not certain that this indicates impairment of definite groups of cells as is the case in some other organs, for we have been unable to discover as many clearly differentiated types of liver cells as there are types of hepatic function.

Crile emphasizes the fact that the important problems in present day abdominal surgery are to be found in the so-called "bad risk" cases, individuals in whom the margin of safety has been reduced by starvation or infection, by the toxins of cancer, by auto-intoxication, etc. He has stressed the importance of exhaustion in predisposing to bad risks, and finds that in all types of experimental exhaustion the cells of the liver show pathologic changes, such as diminished power of differential staining, edema and increased electrical conductivity. He believes that the prognosis is often correlated with the ability or inability of the liver to properly perform its function. Fortunately we are rarely called upon to treat clinical conditions caused by total suppression of hepatic function. It is in further study of derangement of specialized functions of the organ that we may hope for advancement in our present knowledge and methods.

Rather extensive investigations have been made of the relationship between liver function and sugar tolerance. One of the late reports on the subject is by Jacobson. He found that after an Eck fistula operation, experimental animals developed an extremely low tolerance for levulose. Glucose tolerance is but slightly modified after such an operation. The production of alimentary levulosuria is here regarded as evidence of hepatic insufficiency. Galactose has similarly been used as a test of liver function.

Attempts to quantitate hepatic function by the injection of certain dyes as in the dye tests for renal function, have at best been but partially successful. The results are, however, suggestive. The phenoltetrachlorophthalein test has been rather extensively used, but there are distinct disadvantages such as the difficulty of collecting entire specimens of feces, the discomfort caused the patient by catharsis, and the wideness of range in percent of excretion in individuals with apparently normal livers. The first two disadvantages have been eliminated by the use of the duodenal tube.

Goodpasture described, several years ago, a qualitative test which appears to be quite reliable in cirrhosis of the liver but which for some reason is not widely known and has not come into general use. He reported four cases of atrophic hepatic cirrhosis in each of which sterile specimens of venous blood clotted within the normal length of time, but in which the clot redissolved completely within a few hours at body temperature. He designated this phenomenon fibrinolysis. The clotted blood from normal individuals will usually remain for several days at body temperature without redissolving. The fibrinolysis test for cirrhosis of the liver, particularly for atrophic cirrhosis, appears to be quite consistently positive.

Rowntree, Marshall and Chesney have summarized recent knowledge regarding liver function in part as follows: Under clinical conditions a phenoltetrachlorophthalein output of less than thirty per cent with the appearance of the dye in the urine is of unquestionable significance. Low fibrinogen

values are frequently but inconstantly encountered in cirrhosis. The determination of the lipolytic activity of the blood plasma is of little significance. Goodpasture's fibrinolysis test when positive seems to be of undoubted diagnostic importance, indicating cirrhosis of the liver. While theoretically the sugar tests, particularly the levulose and galactose tests, should give reliable information in functional derangement of the liver, practical experience shows that the results are not trustworthy. It is probable that in the metabolism of any kind of sugar too many other organs besides the liver play a part.

The fact that the liver is the chief urea forming organ of the body suggests that a study of the nitrogen partition of the blood might give information of value. There is no constant change in the percentage of blood urea nitrogen, but the amino-acid nitrogen has been found comparatively high in a large proportion of clinical cases. The partition of the urinary nitrogen gives suggestive but variable information. The ammonia nitrogen and the amino-acid nitrogen was definitely increased in most of the cases studied by Rowntree and his associates, particularly in cirrhosis.

Recently Cammidge, Forsyth and Howard have made a further contribution to the study. Sugar is normally present in the circulating blood. Its amount is determined after removing the blood proteins. If this protein free filtrate is hydrolyzed with heat and hydrochloric acid—a procedure which would convert starches, dextrans and the higher polysaccharides into glucose—there results little or no increase in the amount of sugar. Therefore in normal blood there is little or no higher sugar compound capable of producing glucose on hydrolysis. In diseases of the liver or pancreas, however, such substances do apparently exist in the blood. The determination of blood sugar after hydrolysis gives considerably higher values than before. More sugar has been formed. Cammidge and his co-workers choose to call the difference between the ordinary sugar value and the higher value after hydrolysis the “difference value” of the blood. Observations on normal blood have shown that the difference value is very small and is not appreciably influenced by food or by the nature of the diet. In cases of pancreatic disease the difference value is high. This is particularly true in the fasting patient. If a patient with pancreatic disease is absorbing food, the blood sugar rises. The “difference value” then falls, however. In other words, the abnormal substance tends to disappear during the digestion and absorption of food. As the blood sugar falls to normal again after digestion, the “difference value” again rises. This inverse relationship appears to be characteristic of disturbances of pancreatic function.

In hepatic derangements the difference value curve is again said to be characteristic. During fasting it is abnormally high in pancreatic disease, while in liver insufficiency it is at this time within normal limits. Here again the blood sugar rises after a meal, and after about three hours gradually falls to the normal level. The difference value rises likewise but continues to increase for several hours independent of the later fall in sugar concentration, until a level which may be ten or twelve times the highest normal limit

is reached. There is no constant relationship to the blood sugar such as was found in pancreatic disease. Urine curves show similar changes.

The hypotheses suggested by Cammidge and his associates in explanation of the curves are complicated and presuppose a complex harmonic inter-relationship between the liver and the pancreas. There is also considerable discussion of reactions of the higher polysaccharides which at present is purely theoretical. Here again so many organs of the body play a part in sugar metabolism that conclusions must be accepted with considerable caution. However, if the curves of difference value are found to be characteristic in liver disease, the test will be of assistance even though the explanation be questioned. A distinct advantage of the method lies in the fact that it can be carried out entirely with specimens of blood and urine.

Determination of the appearance time of phenoltetrachlorphthalein in the duodenum by means of the duodenal tube, first reported in detail by Aaron, Beck and Schneider, is without doubt a distinct advance, but this method possesses two distinct disadvantages. First, the percent of excretion cannot be determined, and second, disease within the biliary tract may delay the appearance time, thereby vitiating the results.

The same criticism applies to the recent work of Hatiéganu who follows a similar procedure, using indigo-carmin in place of phenoltetrachlorphthalein.

Aub and Means have sought a possible variation in the specific dynamic action of protein in liver disease by determination of the basal metabolism after a high protein meal, but the results were within normal limits. They were led to the conclusion that the liver is either not an important regulator of the metabolic rate, or is adequate for this purpose even when severely diseased.

Widal, Abrami and Iancovescu in an investigation of the functional efficiency of the liver from the point of view of protein metabolism have evolved a very simple test. Their work is based upon two assumed facts. During normal digestion of protein, intermediary products of digestion such as the albumoses are absorbed into the portal circulation together with the amino acid end products. The liver removes these poisonous substances from the blood thereby preventing entrance into the systemic circulation where their presence would otherwise produce anaphylactic manifestations. It is this ability to remove or destroy the poisonous substances absorbed during protein digestion which the authors would measure.

The intravenous injection of peptone produces a rapid blood crisis characterized by leucopenia, fall in blood pressure and decrease in the clotting time of the blood. These authors claim to produce similar changes after shunting the blood past the liver by an Eck fistula type of operation, provided the experimental animal is at the time digesting protein food. The same operation in a fasting animal produces no such blood-vascular reaction. Similar reactions were obtained by intravenous injection of blood aspirated from the portal vein. The normal liver removes these poisonous substances from the portal blood. In hepatic insufficiency, the passing through into the systemic



circulation of such substances causes blood reactions similar to that from peptone.

The test as carried out is very simple. The blood pressure, white count and clotting time are each recorded two or three times at twenty minute intervals on the fasting patient to determine the normal. The patient is then allowed to drink 200 grams of milk. The examinations are then continued at the same intervals. In the normal individual there is little change. The white count either remains normal or an actual leucocytosis develops. The blood pressure shows little change or even rises. In liver insufficiency on the other hand, a rapid fall in white count occurs which usually reaches a minimum at the end of an hour. The blood pressure may fall ten or twenty millimeters. The clotting time becomes reduced. The most constant finding and the only one for which search is made in routine examinations is leucopenia. The cell count often falls to less than half the original figure.

Lactose or butter fat ingested will not produce this reaction, but 8 grams of casein, the amount present in 200 grams of milk, will do so provided the liver is diseased.

By this test the authors have shown mild liver damage following anaesthetization by chloroform or ether, following arsphenamine administration and in chronic alcoholism.

The method appeals because of ease in determination and because of the apparently logical interpretation, in terms of existing knowledge and theories. Certain drawbacks must be considered. The authors obtain a similar reaction in diabetics after the administration of glucose. This reminds us that there are many possible causes for leucopenia and that such a reaction in and of itself is not specific. Moreover they report positive reactions in rather large percentages of cases of pneumonia, tuberculosis, acute and chronic appendicitis, typhoid and paratyphoid fever, nephritis with increased blood urea nitrogen, and other diseases. Their interpretation is that in these diseases there is often mild, clinically latent hepatic insufficiency. Granted that this explanation is the correct one and that the reaction is entirely specific for liver functional changes, we must conclude that the extreme delicacy of the test is somewhat of a drawback to its usefulness. On the other hand this very sensitiveness would make it extremely valuable as a preoperative routine in cases where surgical shock is to be feared or where anaesthetization will be prolonged.

Delprat and Whipple state that the ideal liver functional test would consist in the introduction intravenously of some non-toxic substance which would test by a synthetic demand the functional reserve of the liver (abnormal or normal). The product formed by this synthesis should be obtainable from the blood rather than from the urine, as renal abnormality would not confuse the issue. They report a series of investigations of sodium benzoate and the rate of its synthesis into hippuric acid, but reach no definite conclusions regarding the relative importance of the liver in this synthesis.

The preceding review emphasizes the knowledge that by no one test can we hope to measure the functional capacity of the hepatic parenchyma. The

number and variety of functions performed necessitates the development of methods which will measure the efficiency of representative functions in the various fields. As Delprat and Whipple have said, in the ideal test possible confusion due to renal or biliary tract abnormality must be eliminated, preferably by carrying out the tests on blood alone. But they overlook the importance of the oral administration of test substances to ascertain those functions playing a part in the digestive process. The dye tests for elimination, the fibrinolysis test, the "difference value" tests, the reaction to ingested protein are all of importance. They are all measurements of entirely different functions of the same organ. In each at the present time too many extraneous variables obscure the results. We may confidently expect in the near future suggestions for the study of yet other functions of this versatile organ. It is highly desirable that all of these leads be followed with a view to simplification and to correlation.

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—W. T. V.

### *The Pathogenicity of B. Botulinus*

WE have been uncertain as to whether botulism results exclusively from an intoxication or may be due also to an infection. Several investigators have tried inoculating animals with spores freed from the toxin. It is generally believed, and this belief has been recently confirmed by Dickson, that the toxin is never formed within the animal body. This is supposed to be due to the fact that *B. botulinus* has its optimum growth at from 24° to 28° C. and that it will not form a toxin at the temperature of the human body, 37.5° C. Shippen found that the spores of a strain with which he worked were not pathogenic to rabbits when administrated by the mouth or subcutaneously. Similar results were obtained by Thom, Edmondson, and Giltner, who inoculated animals with spores freed from toxin by washing or by heating. In the Canton, Ohio, case, attention was called to the fact that it is exceedingly difficult to wash away all the toxin from the spores. More recently, Thom and colleagues report that they have succeeded in producing botulism in guinea pigs by feeding massive doses of spores freed from toxin.

Armstrong, Story and Scott obtained only negative results when they endeavored to induce the disease in animals by inoculation with toxin-free

spores. Some time ago Orr, working with a Nevin strain, produced symptoms of botulism in guinea pigs by feeding or injecting toxin-free spores. More recently, Orr\* has confirmed his former findings, and states his conclusions as follows: "The optimum temperature for growth and elaboration of toxin by *B. botulinus* is that of the body temperature, 37° C. *B. botulinus* can be recovered from the internal organs of animals which have been fed or injected with toxic cultures and also with toxin-free spores of this organism. *B. botulinus* under certain conditions, will grow and produce toxin in the body of the guinea pig. Experimental botulism can be produced in laboratory animals by the feeding or injection of massive quantities of toxin-free spores of *B. botulinus*. The presence of toxin produced in the body as a result of growth of toxin-free spores can be demonstrated by the precipitin test as well as by direct toxicity tests. Botulism poisoning in man due to the ingestion of spores is probably very rare, if it occurs at all. The possibility of such occurrence must, however, be considered.

—V. C. V.

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### *Cod Liver Oil in Rickets*

WHILE the discussion concerning experimental rickets continues to be marked by diversity of opinion, all those engaged in this work apparently agree that cod liver oil is of the greatest service both in the prevention and in the cure of this disease. Mellanby has been the strongest and most persistent advocate of the theory that fat-soluble vitamin A is *par excellence* the antirachitic constituent of food. That fats do play an important part in the normal nutrition of cartilage and bone there seems to be no doubt. Paton has quite satisfied himself by experiments on dogs that exercise is an important, if not an essential, factor in preventing rachitic conditions. The discoverer of rickets, so to speak, Glisson, who wrote in the seventeenth century, was quite convinced that sunlight has much to do with the prevention of rickets, and this idea has recently received experimental confirmation at the hands of several reliable observers. Ever since Glisson called attention to the condition of malnutrition of bone and cartilage, which we now designate as rickets, cod liver oil has been used in its treatment. Every now and then during these centuries some one ridicules the use of this medicinal agent, and in one or two instances experiments have apparently proved it to be worthless, but we always come back to the idea that cod liver oil is of value and, indeed, of greater value than any other fat in the treatment of rickets.

Recently, McCollum and colleagues,<sup>1</sup> studying experimental rickets at the Johns Hopkins School of Hygiene, made the following statement: "On a diet such as we have employed, young rats are much better nourished, when supplied with one per cent of cod liver oil than with ten to twenty per cent of

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\*Jour. Infect. Dis., 1922, xxx, 118.

<sup>1</sup>Jour. Biol. Chem., 1922, 1, 6.

butter fat, as is shown by better growth, fertility, success in rearing young, and in length of life. This is true, notwithstanding that three per cent of butter fat is ample for providing the animals with sufficient fat-soluble A and any other organic substance exerting a special effect on the bones, when the content of calcium in the diet is raised to approximately half the optimal. The provision of nearly seven times this amount does not exert much protection to the animals against the specific detrimental effect of lack of calcium when the content of the diet in this element is from one-fifteenth to one-fifth or sixth the optimal amount. One per cent of cod liver oil, on the other hand, seems to increase in a very remarkable manner the effectiveness with which the anatomic elements of the body tissues deal with a very low calcium supply. Our results indicate that there is no progressive benefit to the animals brought about by the exhibition of greater and greater amounts of butter fat. This would indicate that the effects are not proportional to the amount of this fat which is included in the diet. Furthermore, it appears that one to two per cent of cod liver oil supplies as much of the substance which exerts a peculiar influence on bone growth as the animals can profit by, even when the calcium supply is very low indeed. An extensive experience in feeding diets of the type here described has failed to reveal any evidence that a rat is benefited by the inclusion of more than five per cent of butter fat when the calcium and phosphorus are normal. It is further shown from the experimental data recorded in the charts that as the calcium content of the diet is increased the differences between the effects of butter fat and cod liver oil tend to disappear, and vanish completely or nearly so when the content of calcium reaches as much as one-half or more of the optimal amount. The results of this series of experiments were so consistent and decisive that we can deduce no other conclusion than that cod liver oil contains in abundance some substance which is present in butter fat in but very slight amounts, and which exerts a direct influence on the bone development and enables animals to develop with an inadequate supply of calcium much better than they could otherwise do. This substance is apparently distinct from fat-soluble A, which is essential for growth and which is associated definitely with the prevention of ophthalmia (keratomalacia)."

McCollum assures us that he and his colleagues are continuing investigations along these lines and that they hope in the near future to be able to tell us what constituent of cod liver oil is so effective in bone nutrition when the amount of calcium supplied in the food is dangerously low.

—V. C. V.



# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, JULY, 1922

No. 10

## ORIGINAL ARTICLES

### STUDIES IN FOCAL INFECTION\*

#### PRELIMINARY REPORT

BY R. N. DE NIORD, M.D., F.A.C.P., AND B. J. BIXBY, M.D., BUFFALO, N. Y.  
DIAGNOSIS

MANY excellent papers have been written and much scientific research work has been carried on by scores of investigators in the effort to determine and to clarify the relationship of focal infections to systemic disease. Due to the preponderance of evidence, the facts which have been adduced should be fully accepted by both the medical and dental professions. But unfortunately there are many in both professions, who have failed to accept the so-called "focal infection theory" either in whole or in part. The reasons for this lie in prejudice, lack of ability to draw conclusions from the overwhelming facts and data already presented, or unfortunate failures in practice due to incomplete search for and eradication of primary foci. We propose to present in this paper a broad and yet concise view of focal infection as a source of systemic disease, and particularly to present an invariable diagnostic factor by which the presence of focal infection may be determined.

The subject of focal infection is not new. As early as 1789, Eyerlin of Christiana in his *Materia Rheumatica ad Tonsillitis*, considered the relationship of diseased tonsils to rheumatism as clinically evident. In 1818, Benjamin Rush<sup>1</sup> laid before the American Society of Ophthalmology and Otolaryngology at a meeting in Cleveland, Ohio, a case of rheumatism of the hip accompanied by toothache, the rheumatism being promptly cured by extraction of a decayed tooth. From Rush's paper in 1818 up to 1909, a review of medical literature reveals the fact that no scientific proof of the connection between focal infection and systemic disease had been published.

\*Received for publication, May 10, 1922

<sup>1</sup>Medical Inquiries, Benjamin Rush, 1818.

Nevertheless it was clinically evident that certain infectious diseases followed tonsillitis. And it was furthermore shown that the same organisms were present in the tonsils and in the secondary lesion; up to this time the clinical evidence was so positive and even overwhelming, that scientific proof seemed scarcely necessary. In spite of this evidence, however, the majority of the medical profession remained sceptical up to a few years ago. During the past ten years, however, many scientists working independently and in different fields, have presented such an abundance of evidence that it would seem that the truth of the thesis had been established, particularly as the data were based on both clinical evidence and experimental and research work. In spite of this there is a large percentage of both medical and dental professions who are still disposed to doubt. And it is with the hope of removing this scepticism and to establish a standardized routine examination by which the presence of focal infection may be detected, that the following studies of changes in the chemistry of the blood are presented. We wish to express our appreciation of the efforts of the American workers, which have made this study possible, such men as Folin, Benedict, Meyers, Van Slyke, and others. We present here nothing new as to methods of examination, but we do present a new interpretation of the findings brought out through the results of their work. It has been found in a series covering several hundred cases, that certain changes invariably take place in the blood chemistry of patients having a focal infection. The findings in these cases may be modified to a certain extent by previous medication and dietary restrictions prescribed for the most part by careful observers working from an empirical standpoint. But when these forms of treatment are known to have been given, the diagnosis may still be made with a high degree of accuracy.

Under the heading of Focal Infection may be included chronic accumulations of pus, and areas exhibiting an abnormal degeneration of the cellular elements, enclosed in any of the cavities or tissues of the body. Most prominent among these tissues are the tonsils, including all lymphoid tissues in Waldeyer's ring, teeth and periodontal tissues, nasal accessory sinuses, middle ear, mastoid and lateral sinuses: in fact all cavities communicating with the mouth, nose, and nasopharynx. The consensus of opinion seems to give the tonsils the place of primary importance as foci of infection, with the teeth and periodontal tissues as second in importance. The list of the most common diseases in which focal infection may prove to be of paramount etiological significance are as follows:

- |                       |                       |
|-----------------------|-----------------------|
| 1. Neurasthenia       | 10. Arthritis-chronic |
| 2. Neurosis           | 11. Gout              |
| 3. Insanity (Cotton)  | 12. Osteitis          |
| 4. Neuritis           | 13. Periostitis       |
| 5. Neuralgia          | 14. Basedow's disease |
| 6. Myalgia            | 15. Chorea            |
| 7. Myositis           | 16. Meningitis        |
| 8. Arthritis-acute    | 17. Septicemia        |
| 9. Arthritis-subacute | 18. Furunculosis      |

- |                        |                                  |
|------------------------|----------------------------------|
| 19. Herpes zoster      | 35. Gastric ulcer                |
| 20. Urticaria          | 36. Duodenal ulcer               |
| 21. Erythema nodosum   | 37. Appendicitis                 |
| 22. Myocarditis        | 38. Colitis                      |
| 23. Endocarditis       | 39. Cholecystitis                |
| 24. Pericarditis       | 40. Cholangitis                  |
| 25. Arteriosclerosis   | 41. Daeroecystitis               |
| 26. Bronchitis         | 42. Conjunctivitis               |
| 27. Pneumonia          | 43. Iritis                       |
| 28. Asthma             | 44. Corneal ulcer                |
| 29. Diabetes-mellitus  | 45. Retinal hemorrhage           |
| 30. Diabetes-insipidus | 46. Optic neuritis               |
| 31. Nephritis          | 47. Diplopia                     |
| 32. Pyelitis           | 48. Glaucoma (?)                 |
| 33. Cystitis           | 49. Possible adjuvant in produc- |
| 34. Gastric neurosis   | tion of cancer.                  |

Regarding the last named possible effect of focal infection it has been noted that an infected mouth frequently shows a tendency to an acid reaction, this acid reaction favoring cell degeneration of a malignant type following chronic irritation (Mayo).

Rosenow, who has done so much to establish the relationship between focal infection and systemic disease, has said, "In the light of our present knowledge, the argument that infections in the mouth are so common in the mouths of individuals in apparent health, does not minimize their importance. These or other foci are so common in patients suffering from arthritis, neuritis, appendicitis, ulcer of the stomach, cholecystitis, goiter, etc., and so rare in individuals who have had superb health for years that their direct etiologic rôle can scarcely be questioned. The most common location of the focus or source of infection is in the mouth, and the teeth as well as the tonsils must always be carefully examined with this object in mind. However the question of the focus of infection is a matter not only for the stomatologist or the dentist, but also for the general practitioner, the internist and the surgeon. In fact, every branch of medicine needs to be taken into consideration in order to trace the focus from which the organisms gain entrance to the body. The focus of infection must be regarded not only as a place of entrance for the bacteria, but also the place where the organisms acquire the peculiar properties necessary to infect."

The diagnosis of these areas frequently offers one of the most difficult problems in medicine. We find, for instance, many evidences of infection in the mouth in areas whose degeneration is not sufficiently pronounced to be macroscopic,—which have not reached the stage of definite abscess formation with a well-defined walled-off area of pus. Such areas, however, invariably present microscopic evidence of inflammatory changes. The bone may exhibit rarefaction, or condensation, or may only show a change in the arrangement of the cancellous spaces. Their variation in density or structure from the normal bone surrounding them may be so slight as to readily escape

detection. To our minds these areas are even more dangerous than the areas marked off by a layer of dense bone or a pyogenic membrane, as they allow toxins to be absorbed freely into the circulation. These areas of diffuse rarefying osteitis and diffuse condensing osteitis, or the areas exhibiting an alteration in the cancellation of the bone, produce virtually the same leucocytic infiltration, the same degeneration of tissue and blood elements, the same liberation of split proteins, as does the well-defined abscess. The toxic effects of split proteins need not be taken up here: it has been thoroughly investigated by Victor C. Vaughan, ("Poisonous Proteins"). Too often has disappointment for both practitioner and patient followed a diagnosis which was incomplete rather than incorrect. For not only in the mouth, but also in other parts of the body, is a definite diagnosis very difficult to make. And it is safe to say that the major part of the present scepticism regarding the focal infection theory rests on this point.

The blood is the one tissue of the body which is in intimate contact with all the other tissues. Into it are eliminated all the products of the breaking down of tissue, both the normal katabolic end products and those substances which are produced as the result of disease. To the blood, even more than to the urine, we must look in our study of the body, whether in health or disease. The tissues of the body including the blood, consist of cells and intercellular substance. When any tissue breaks down, either completely or in degenerative change, there is an abnormal liberation of the chemical units of which that tissue is made up, each tissue and its parts having their characteristic chemical units. However, all cells no matter what their composition, have nuclei which have the same chemical foundation, in that they consist largely of nucleo-proteins. These are conjugated proteins, being compounds of one or more proteins with nucleic acid. Nucleic acid upon decomposition yields phosphorus and purine bases. In the body the usual end-product of the decomposition of nucleo-protein is uric acid, which is usually classified as *endogenous*, originating in the breaking down of the nuclear portion of tissue elements, and *exogenous* derived from the breaking down of ingested food containing the purine nucleus. A further subdivision is now found necessary for the endogenous uric acid, namely, *normal*, that originating in normal tissue metabolism, and *abnormal*, that originating in nuclear degeneration. Other nitrogenous end-products of metabolism or of tissue degeneration are urea, ammonia and creatinine. And it is interesting to note that each of the four nitrogenous end-products has a different threshold of elimination by the kidney. At present, however, we will consider only that substance, which is of nuclear origin; namely, uric acid, remembering that its presence in the blood may be traced to the normal metabolic processes, ingestion of food rich in purin bodies, or the abnormal breaking down of cellular elements. The main fact to which we wish to call attention is that infections, whether producing pus or not, give rise to a nuclear degeneration of a proteolytic type from the nuclei of the cells so broken down, the end-product of which is uric acid.

Uric acid is eliminated with some difficulty by the kidneys and consequently tends to accumulate in the blood when produced in larger quantities



TABLE I

The cases so reported in this series are those in which the Urea Nitrogen—Total non-proteid Nitrogen and Creatinine fell within the normal limits, the Urine findings running parallel with the blood in that there was no gravity fixation and at the most showed a faint trace of Albumin. We will report therefore at this time only the Uric Acid findings.

NAME	AGE	SEX	URIC ACID IN MGMS. PER 100 C.C.	DIAGNOSIS	TREATMENT	RESULTS AND URIC ACID FINDINGS	
E. T. S.	34	M	4.3	Erythema nodosum	A. Tooth extraction B. Tonsillectomy	Improved Recovered	3.2 1.6
B. J.	56	F	3.2	Sciatica	Tooth extraction	Recovered	1.4
J. Me.	34	F	3.5	Arthritis (sub-acute) Neurosis	A. Tooth extraction B. Appendectomy	Improved Recovered	3.1 1.5
C. M. J.	47	M	3.7	Psychoneurosis	Tooth extraction	Recovered	1.5
F. H.	40	F	4.5	Beginning Glaucoma	Tooth extraction	Recovered	1.6
A. R.	31	F	3.9	Corneal ulcer	A. Tooth extraction B. Tonsillectomy	Improved Recovered	3.1 1.7
R. J.	59	F	3.7	Intercostal neuralgia	A. Tonsillectomy B. Tooth extraction	Improved Recovered	3.1 1.5
N. A. T.	44	F	4.2	Gout	Tooth extraction	Recovered	1.6
M. B.	14	F	3.4	Chorea	Tonsillectomy	Recovered	1.6
C. J.	45	M	4.1	Debility and neurosis	A. Tooth extraction B. Appendectomy	Improved Recovered	2.8 1.4
J. K.	31	M	3.8	Neuritis and psycho- neurosis	A. Tonsillectomy B. Tooth extraction	Improved Recovered	2.4 1.6
E. K.	43	F	4.3	General neuritis and neurosis	A. Tooth extraction B. Tonsillectomy C. Cholecystectomy	Improved Improved Recovered	3.4 2.4 1.4
I. T.	41	F	3.7	Arthritis (sub-acute)	A. Tooth extraction B. Tonsillectomy C. Fistula in Ano	Improved Improved Recovered	3.4 2.6 1.5
F. A. H.	33	M	3.6	Neurasthenia	Tooth extraction	Recovered	1.3
C. P.	35	M	3.8	Myalgia	Tooth extraction	Recovered	1.2
A. Me.	37	F	4.2	Arthritis	A. Tooth extraction B. Tonsillectomy	Improved Recovered	3.7 1.6
L. L.	23	F	3.4	Arthritis and neurosis	A. Tooth extraction B. Tonsillectomy	Improved Recovered	3.1 1.4
D. L. J.	38	F	4.2	Cervical neuralgia	Tooth extraction	Recovered	1.6
J. S.	57	M	4.1	Psychoneurosis and Myocarditis	Tooth extraction	Recovered	1.3
W. H. D.	61	M	3.7	Optic Neuritis and Myocarditis	Tooth extraction	Recovered	1.2
H. H.	46	F	3.3	Neuritis and second- ary anemia	A. Tooth extraction B. Tonsillectomy	Improved Recovered	2.5 1.4

than normal over a period of time. Examination of the urine may thus for a long period of time show no more than the normal content of uric acid, while at the same time the blood chemistry reveals a uricacidemia.

It must, of course, be kept in mind that other agencies than chronic infections may produce uricacidemia, as for instance leukemia, primary anemia, cachexias, massive doses of x-ray and radium, etc. All of these conditions are susceptible of diagnosis and elimination. And when so eliminated the presence of a high uric acid content in the blood may safely be taken as indicating that somewhere in the body there is in progress an unusual breaking down of nuclear material. All chronic infections are divided into primary and secondary. And while the secondary infection is usually the source of anxiety to the patient, the primary or inaugurating infective focus is the point of interest to the diagnostician. The presence of a high blood uric acid does not of course point to any particular part of the body as its source. Hence the diagnosis of focal infection made through this medium does not indicate that the teeth or tonsils or any other one organ is at the bottom of the disturbance. But it does point to the necessity for a thorough search for all possible primary foci, and it gives definite authority for the removal of such foci when found.

Practice based upon this assumption has been most happy in the results which it yields. Patients invariably show such a measure of improvement as is to be expected according to their history, and the regenerative power of the tissues which are the site of the secondary infections. Moreover when improvement does not occur, or is incomplete, the possible continuance of focal infection will be revealed by a continued high uric acid in the blood. For the uric acid will not return to its normal level in the presence of primary foci of infection, and on the other hand when these foci are completely eliminated, the uric acid content of the blood will be found to come with the normal range.

#### CONCLUSIONS

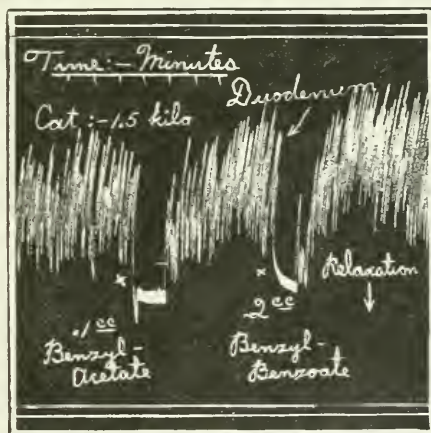
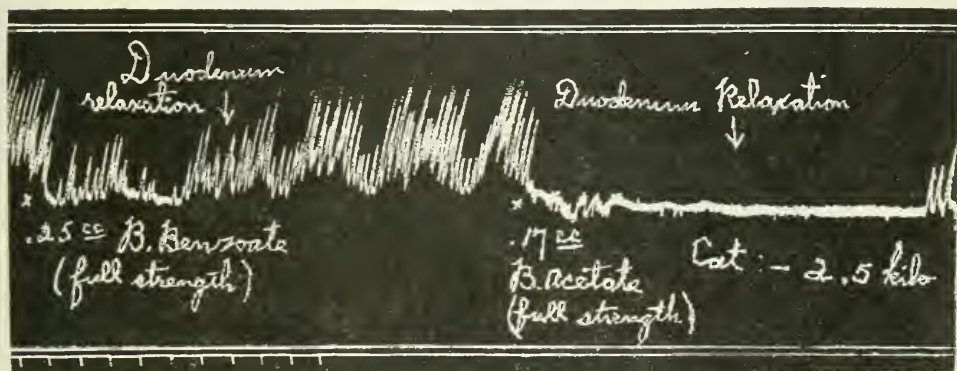
E 2

1. Clinicians have at their disposal well established methods of the estimation of uric acid.
2. High uric acid value in the blood is indicative of nuclear degeneration, which in turn may mean focal infection.
3. Other factors productive of high uric acid, aside from nuclear degeneration, are comparatively easy to determine, i.e., leukemia, primary anemias, cachexias from whatever cause, and massive doses of x-ray or radium.
4. Elimination of all foci of infection invariably is followed by a return of the uric acid to normal.
5. Failure to eliminate all foci will prevent the return to a normal uric acid, and this substance therefore furnishes a reliable index to the complete elimination of foci of infection.

## FURTHER OBSERVATIONS OF THE PHARMACOLOGY OF BENZYL COMPOUNDS.\* II.

BY CARL NIELSEN AND JOHN A. HIGGINS, CHICAGO, ILL.

IN the course of our pharmacologic investigations of benzyl compounds we have endeavored to establish the relative efficiency of various benzyl esters and other benzyl compounds as intestinal relaxants. Our work has included benzyl esters of melting points both below and above room temperature.



Figs. 1 and 2.—Show a comparative action of benzyl benzoate and benzyl acetate on the cat's duodenum.

These were all supplied by the Chemical Research Department of The Abbott Laboratories, and have been described chemically by Volwiler and Vliet.<sup>1</sup> In a previous article<sup>2</sup> we presented records obtained with liquid benzyl esters mainly, pointing out that our results suggest a correlation of rate of benzyl

\*From the Pharmacologic Department of The Abbott Laboratories, Chicago.  
Received for publication, December 15, 1921.

hydrolysis and smooth muscle relaxant property in the cases of simple benzyl esters. In further support of this theory, we present records (Figs. 1 and 2) of comparative action on the cat's duodenum of benzyl benzoate and benzyl acetate. In Fig. 1 the doses injected were equal in benzyl content; in Fig. 2

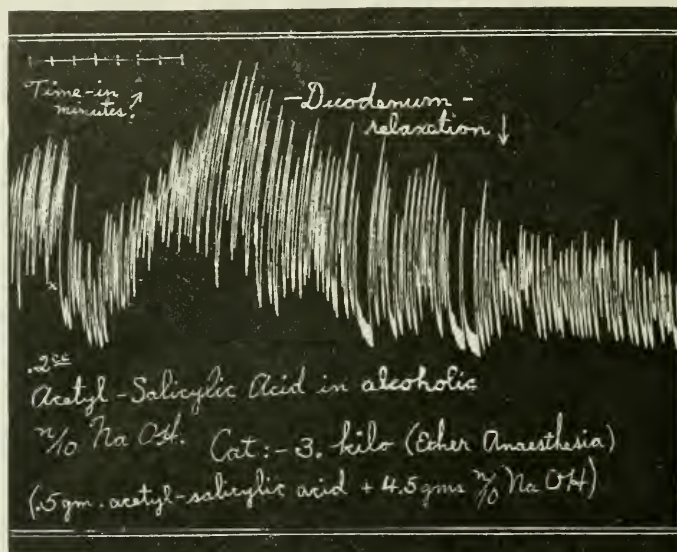


Fig. 3.—Shows the action of acetylsalicylic acid on the duodenum of a cat.

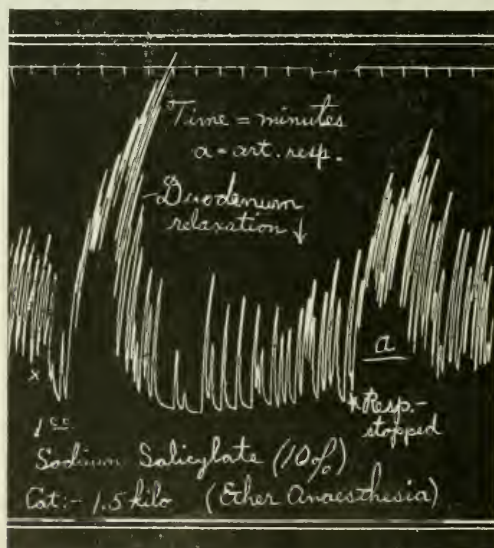


Fig. 4.—Shows the action of sodium salicylate on the duodenum of a cat.

the dose of benzyl acetate was one-half by volume of that of benzyl benzoate.

Benzyl salicylate and benzyl acetylsalicylate, which both contain substituting groups, appear as exceptions to this rule. These two esters were found to produce a relaxation of the intestine, *in situ*, greater than expected from their benzyl content and rate of benzyl hydrolysis.



We have, therefore, carried out a series of experiments to determine whether the relatively powerful relaxant action on smooth muscle of these two benzyl esters may be ascribed in part to their acid radical. Our method of procedure was, to test the action of other salicylates, as well as acetyl-salicylic acid, upon the intestine *in situ*, following the same technic as that used in the case of the esters.

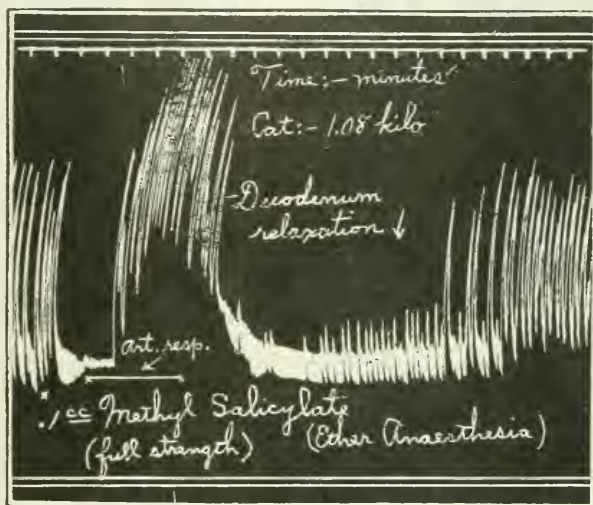


Fig. 5.—Shows the action of methyl salicylate on the duodenum of a cat.

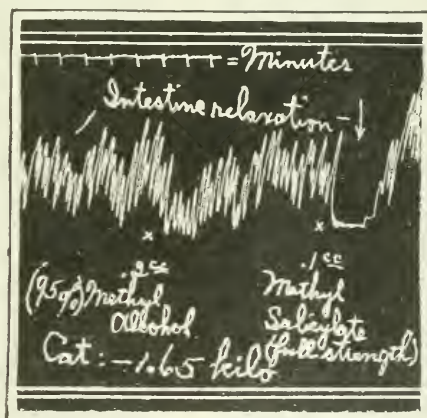


Fig. 6.—Shows a comparative action of methyl alcohol and methyl salicylate on a cat's intestine.

Fig. 3 shows the result obtained on the duodenum of a cat from the injection of 0.2 c.c. of a 10 per cent solution of acetylsalicylic acid in tenth-normal alcoholic sodium hydroxide. The contraction which followed the immediate primary relaxation was probably due to the depression of respiration. As the respiration returned to normal, it will be noted that a secondary and more lasting relaxation appeared. The amount of alcohol injected doubtless interfered somewhat with the reaction, but it was nevertheless chosen as a solvent because of the low solubility of acetylsalicylic acid in oil.

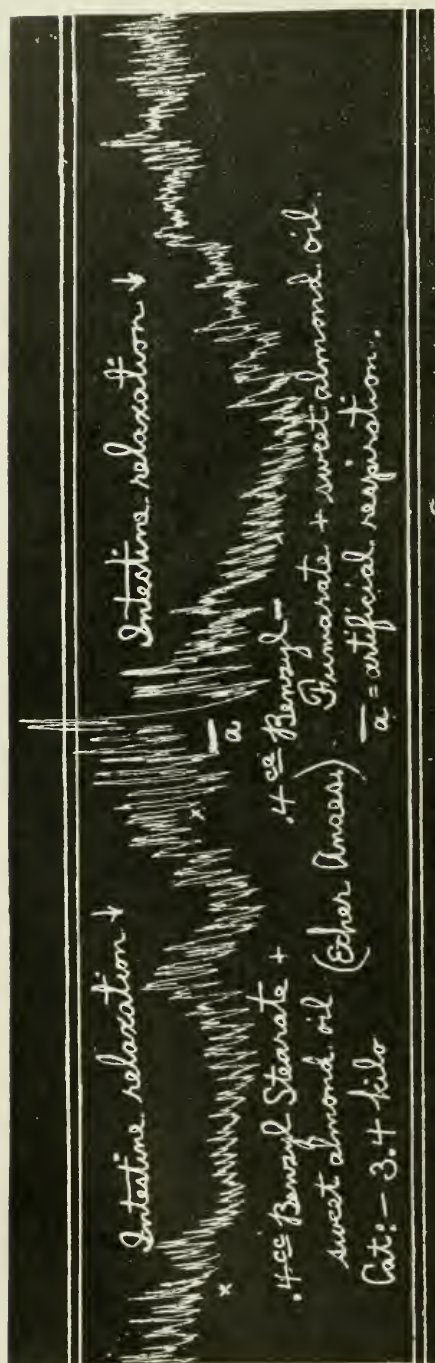


Fig. 7.—Shows a comparative action of benzyl stearate and benzyl fumarate on the intestine of a cat.

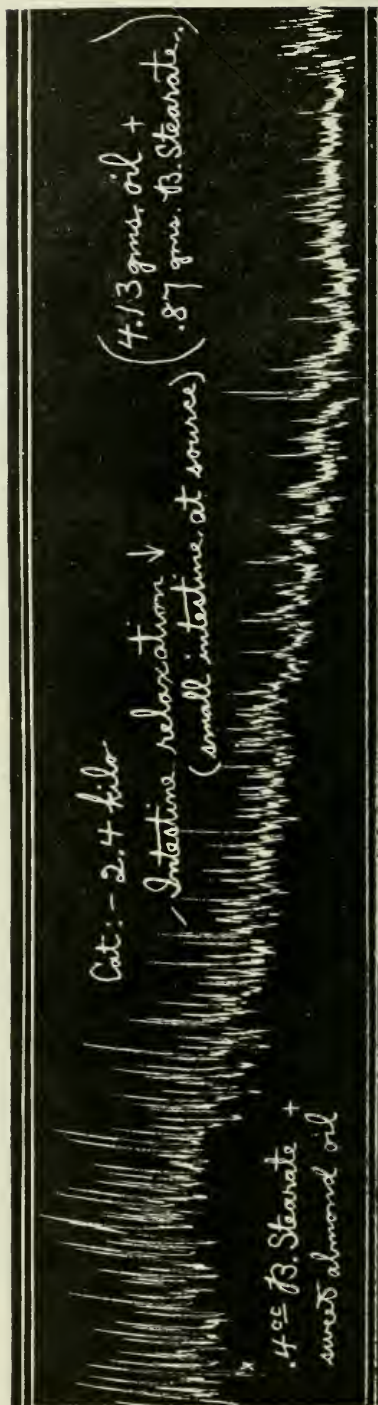


Fig. 8.—Shows the action of benzyl stearate on longitudinal muscles of a cat's duodenum.

Fig. 4 demonstrates the action on the duodenum of a cat of 1 c.c. of a 10 per cent sodium salicylate. Here again we note a primary relaxation followed by a contraction due to respiratory depression, and with the gradual return of normal respiration, a secondary relaxation of the duodenum.

Fig. 5 shows the effect on the duodenum of a cat from an injection of 0.1 c.c. of methyl salicylate. Since the relaxation produced by this compound was so pronounced, a test was made to compare the action of methyl alcohol with that of methyl salicylate.

Fig. 6 shows that, while methyl alcohol did relax the intestine, the methyl salicylate, containing less methyl in the molecule, produced a decidedly greater relaxation.

Benzyl salicylate, when treated with alcoholic potassium hydroxide, in

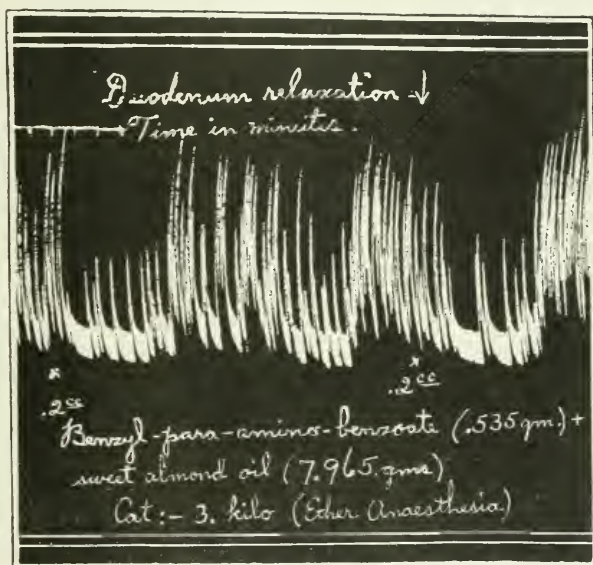


Fig. 9.--Shows the action of benzyl para-amino benzoate on the duodenum of a cat.

vitro, has the lowest rate of hydrolysis of all the benzyl esters we have investigated. The rate of hydrolysis of the benzyl group in benzyl acetylsalicylate does not exceed that of the salicylate (See Volwiler and Vliet, Journal of American Chem. Soc., 1921, 43, 1672). Since their power to relax smooth muscle of the intestine is greater than that of simple benzyl esters possessing a much higher rate of hydrolysis, it appears reasonably certain that this greater efficacy is due to their difference in structure from the simple benzyl esters. This difference consists in the molecule of the salicylate containing a hydroxyl group and that of the acetylsalicylate, a substituted hydroxyl group. Our records demonstrate, on the other hand, that these acids alone do not possess any striking power to relax the intestine. It seems evident, therefore, that the action of these two benzyl esters is not the ensemble of their molecular constituents, but rather a more potent one possessed by the intact molecules.



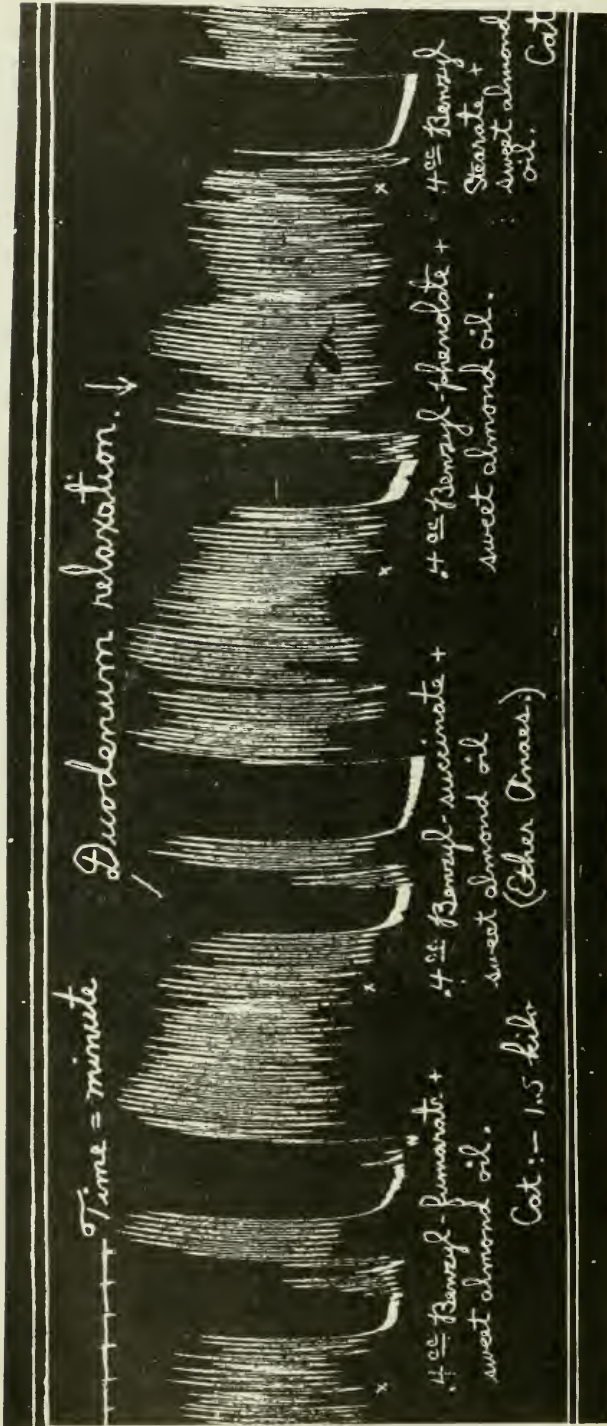


Fig. 10.—Shows a comparative action of various solid benzyl compounds on the duodenum of a cat.



In our investigation of benzyl esters of higher melting points (solid above room temperature), we have used the Trendelenburg method,<sup>3</sup> unless otherwise specified. The benzyl esters were dissolved in sweet almond oil in such amounts that the various solutions used for injection contained the same proportion of the benzyl radical. Thus, for instance, 0.87 gm. of benzyl stearate dissolved in 4.13 gm. of oil correspond in benzyl content to a solution of 0.35 gm. of benzyl fumarate in 4.65 gm. of oil.

Fig. 7 presents a comparison between benzyl stearate and fumarate on the intestine of a cat. It shows that benzyl fumarate produces a greater relaxation than benzyl stearate when injected in less than half the amount by weight.

Fig. 8 is a record taken by the Jackson method,<sup>4</sup> showing the rather

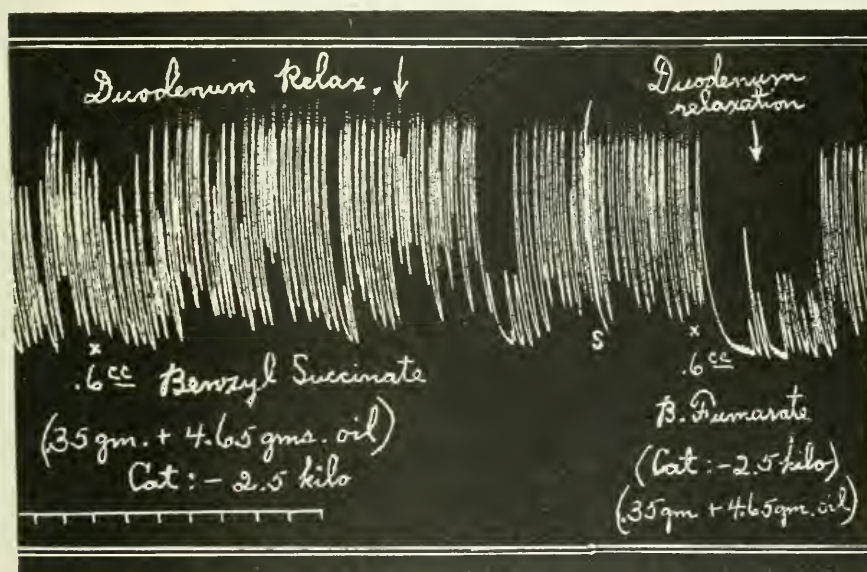


Fig. 11.—Shows a comparative action of benzyl succinate and benzyl fumarate on the cat's duodenum.

slow relaxation of the small intestine of a cat produced by benzyl stearate.

The action of benzyl para-amino-benzoate on the cat's duodenum is shown in Fig. 9. This ester was a fawn-colored crystalline powder, melting between 88.5 and 89.5° C. It was first dissolved in the proportion of 0.535 gm. in 4.465 gm. of oil, to conform in benzyl content with the other benzyl ester solutions, but in this proportion, it did not remain in solution at body temperature. An addition of 3.5 gm. of oil was therefore made.

In our study of comparative efficiency of the solid benzyl compounds, we have had continuous evidence of the regrettable fact that these compounds cannot be administered intravenously in a solvent more suitable than oil. The cat, which we have found to be the animal best suited for our work, naturally presents differences in individual response to this mode of administration. As a rule, the younger animals react more readily than the older. Fig. 10 is a typical example of a readily responding duodenum in a young cat.

The solutions injected were made to conform in benzyl content, with the exception of the benzyl phenolate which contained 1 gm. plus 4 gm. of oil. The relaxation of the duodenum took place only 2 to 3 minutes after each injection, regardless of any differences in the rate of initial hydrolysis of the various



Fig. 12.—Shows the action of a 5 per cent solution of mono-benzyl barbituric acid on the duodenum of a cat.

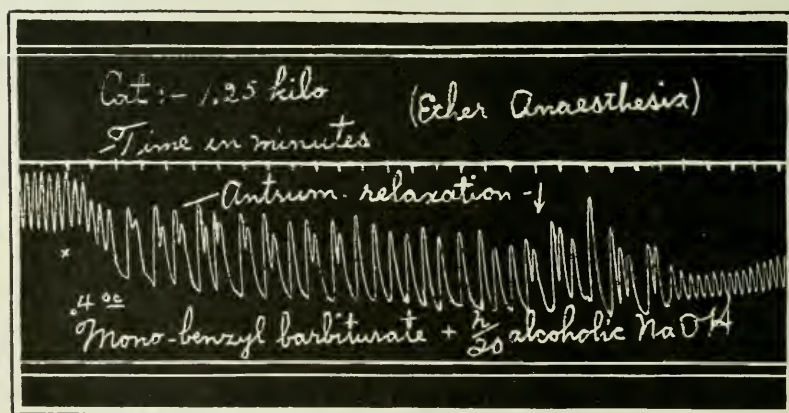


Fig. 13.—Shows the action of a 2 per cent solution of mono-benzyl barbituric acid on the antrum pylori of a cat.

compounds. The recovery was rapid in all instances; the slight difference in the duration of relaxation being the only means of comparison.

As a typical contrast to this tracing we present Fig. 11. This cat was an old female. An injection of 0.042 gm. of benzyl succinate in oil was made. Approximately fifteen minutes elapsed before the first sign of relaxation appeared. The drum was stopped at S. for a period of 45 minutes, after which

time the intestine had recovered from a series of alternate relaxations and contractions. An injection of the corresponding benzyl molecular amount of benzyl fumarate (0.042 gm.) in oil was now made and produced a relaxation in less than one minute. This difference in onset of relaxation corresponds to the difference in the rate of initial hydrolysis *in vitro* of these two esters. Volwiler and Vliet show that benzyl succinate hydrolyzes at the rate of 12 per cent in 5 minutes, 16.8 per cent in 15 minutes and 17.1 per cent in 20 minutes; while benzyl fumarate hydrolyzes at the rate of 35 per cent in 5 minutes, 41.5 per cent in 15 minutes, and 43 per cent in 20 minutes when treated according to their method. After a period of two hours their rates of hydrolysis run closer together, namely, 40 per cent for the succinate and 57 for the fumarate.

We obtained also marked relaxations of the cat's duodenum with other benzyl compounds. The records obtained with benzyl phenolate have already been presented. Benzaldehyde was also included in our experiments.

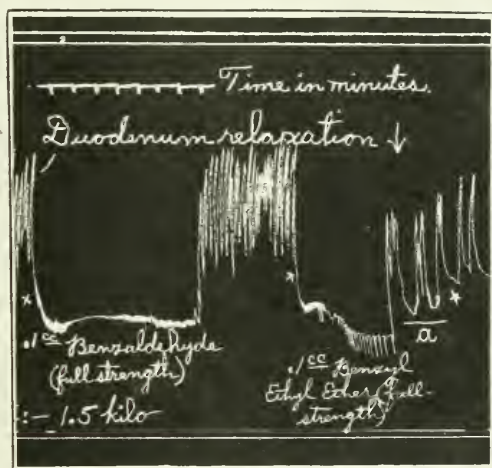


Fig. 14.—Shows the action of benzaldehyde and benzyl ethyl ether on the duodenum of a cat.

Figs. 12 and 13 show the action of monobenzyl-barbituric acid on the duodenum and antrum pylori of a cat. It produced a relaxation of both. On the antrum, the relaxation was preceded by an increase of vigor and decrease of frequency of the movements. The antrum experiment was made according to the Jackson method.<sup>4</sup>

Fig. 14 shows the action of benzaldehyde and benzyl ethyl ether on the duodenum of a cat. The benzyl ethyl ether has a marked depressing effect on the respiration, even upon inhalation. Injected intravenously, it stopped the respiration and produced a gasping effect. This gasping may be noticed in the tracing by the downward strokes of the lever after the duodenum had relaxed. Artificial respiration was applied but failed to revive the animal, the heart having stopped.

Fig. 15 shows the relaxation of the isolated uterus of a virgin guinea pig produced by benzyl ethyl ether and benzaldehyde.



## CONCLUSIONS

Our observations suggest that the relaxing power of benzyl esters on the smooth muscle fibers of the intestine as a rule is dependent upon the benzyl content and upon the rate of hydrolysis of the benzyl compound. In all cases where we have succeeded in obtaining comparative records, with the exception of those of benzyl salicylate and benzyl acetylsalicylate, we have found that the higher the rate of hydrolysis, the greater the efficiency. According to our findings benzyl fumarate is more efficient than benzyl succinate, particularly with regard to initial action; the succinate is more efficient than the stearate, the acetate more powerful than the cinnamate, which in turn is of higher efficiency than the benzoate.

The high relaxing power of benzyl salicylate and benzyl acetylsalicylate, in spite of their slow rate of benzyl hydrolysis, may be attributed to their

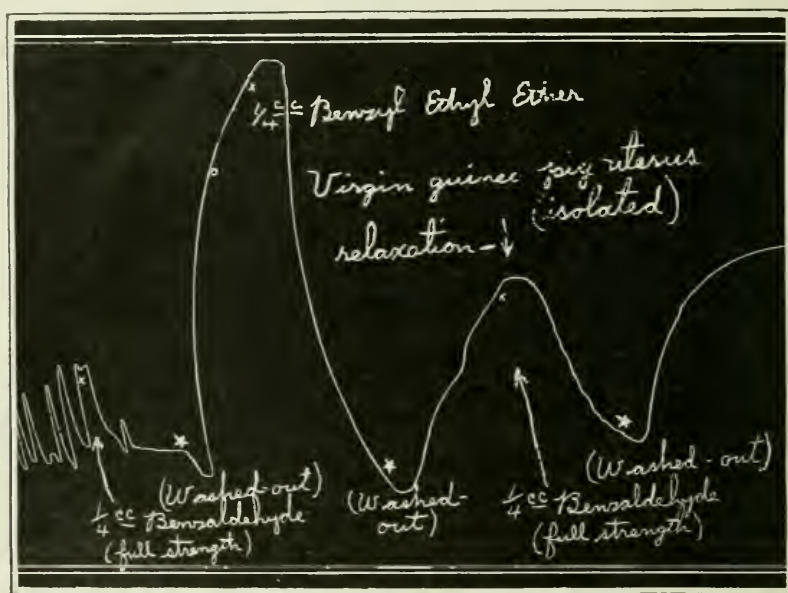


Fig. 15.—Shows the action of benzaldehyde and benzyl ethyl ether on the isolated uterus of a virgin guinea pig.

intact molecules. These compounds differ from other benzyl esters in that their molecules contain hydroxyl or substituted hydroxyl groups. The benzyl acetylsalicylate is more efficient as an intestinal relaxant than the salicylate, in fact it is more powerful than any of the benzyl esters investigated by us.

Various benzyl compounds other than benzyl esters, namely benzyl phenolate, benzyl ethyl ether, and monobenzyl barbituric acid, as well as benzaldehyde, were also found to possess smooth muscle relaxing properties.

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## THE PRESENCE OF BACTERIA AND FORMED ELEMENTS IN THE URINE OF RABBITS\*

BY HENRY F. HELMHOLZ, M.D., AND FRANCES MILLIKIN, ROCHESTER, MINN.

THE occurrence of so-called spontaneous nephritis in rabbits has often been commented on by investigators who use these animals for experimental purposes. Bell and Hartzell have accurately described the pathogenesis of the lesions and have emphasized the necessity for adequate controls when rabbits are used in the study of nephritis. They call attention to the fact that some groups of rabbits may be entirely free from evidences of nephritis, while other groups are affected. In eight instances they attempted to isolate the etiologic organism by culturing pieces of kidney. Cultures from four of the kidneys were negative, two yielded a streptococcus and two a staphylococcus. These organisms, however, when injected intravenously into rabbits, failed to reproduce the disease. No other bacteriologic work was done, the discussion being confined chiefly to the histology and pathology of the affected kidneys.

Helmholz and Beeler, in experiments on urinary infections in rabbits by *Bacillus coli*, found one rabbit with a naturally acquired pyelocystitis. The urine contained pus, and cultures yielded *Bacillus coli communis*. It is worthy of note that this was the only rabbit of a large series whose urine contained bacteria, pus, or other pathogenic elements. The colon bacillus isolated in this case was the one used in subsequent experiments.

Recently, we decided to continue the experiments, which were interrupted by the war, and began to search for another rabbit whose urine contained pus and colon bacilli. Contrary to previous experiences, the urine of a large number of rabbits was found to contain pus, erythrocytes, and albumin or casts associated with bacteria, usually colon bacilli. Furthermore, in accordance with observations of Bell and Hartzell, the condition was more prevalent among certain groups; occasionally, lots were entirely free from urinary indications of pathologic conditions. Necropsies performed in a few instances in which urinary findings had been positive, disclosed lesions of the kidney of the type described by Bell and Hartzell.

Since the more or less common occurrence of colon bacilli in the urine of rabbits introduces an important source of error into experiments on the production of urinary infections by that organism, it has seemed worth while to present some of our observations. These serve to emphasize further the necessity of making careful controls, and of selecting a healthy stock with which to work.

\*From the Department of Pediatrics, Mayo Clinic, Rochester, Minnesota.  
Received for publication, December 22, 1921.

TABLE I  
BACTERIA AND FORMED ELEMENTS IN URINE WHICH YIELDED A POSITIVE CULTURE

RABBIT	SEX	LEUKOCYTES IN EACH FIELD (low power)	PUS	OTHER URINARY FINDINGS	FINDINGS FROM CULTURES ON			ORGANISMS IN EACH c.c.
					DEXTROSE BRAIN BROTH	BLOOD AGAR PLATES	LITMUS LACTOSE AGAR	
A-12	M	Very few		Many erythrocytes and epithelial cells	<i>Bacillus coli</i> and streptococcus		Diplococcus <i>Bacillus coli</i>	Few
A-18	F	Very few	+		Diplococcus			
A-19	M	Large clusters of 500			<i>Bacillus coli</i>		<i>Bacillus coli</i>	160
A-24	F	200	+		<i>Bacillus coli</i> and streptococcus		Negative	
A-26	M	8 to 10			Streptococcus		<i>Bacillus coli</i>	100
A-46		100	+	Many epithelial cells	<i>Bacillus coli</i>	<i>Bacillus coli</i>		300
A-48		Large clusters of 200	+		<i>Bacillus coli</i>		<i>Bacillus coli</i>	30
A-50	F	Few clusters of from 8 to 10	+		<i>Bacillus coli</i>	Negative		
A-51	F	Very few		Few erythrocytes	<i>Bacillus coli</i>	Negative	Negative	60
A-55		0		Few granular casts	<i>Bacillus coli</i>	Negative	<i>Bacillus coli</i>	1 per 10 c.c.
A-64		Very few		Many erythrocytes	<i>Bacillus coli</i>	<i>Bacillus coli</i>	and diplococcus	2 per 10 c.c.
A-65	F	Few clusters of from 2 to 3	+		<i>Bacillus coli</i>	<i>Bacillus coli</i>	<i>Bacillus coli</i>	
A-69	F	8	?		<i>Bacillus coli</i>		<i>Bacillus coli</i> Contamination	
A-70	F	0	+		Green-forming diplococcus	<i>Bacillus coli</i>	<i>Bacillus coli</i>	
A-73	F	5			<i>Bacillus coli</i>	<i>Bacillus coli</i>	<i>Bacillus coli</i>	
A-75	F	Few clusters of 25	+		<i>Bacillus coli</i>	<i>Bacillus coli</i>	<i>Bacillus coli</i>	
A-79	F	Few			Diplococcus	Negative	<i>Bacillus coli</i>	Many
*A-79 (one day later)	F	Clusters of 2 to 3			<i>Bacillus coli</i>	<i>Bacillus coli</i>	<i>Bacillus coli</i>	
A-80	F	0			Staphylococcus	Negative	Negative	
A-89	F	Few			<i>Bacillus coli</i>	<i>Bacillus coli</i> and staphylococcus	<i>Bacillus coli</i>	150
A-90	F	Few			Staphylococcus and diplococcus	diplococcus	<i>Bacillus coli</i>	130

\*Necropsy examination revealed a faint red streak in the medulla of the right kidney and *Bacillus coli* in both kidneys.

We examined the urine of ninety-nine rabbits. Pus was found in thirty, casts in three, erythrocytes in three, and colon bacilli not accompanied by any of the foregoing pathogenic findings, in three. The urine of sixty-three of the ninety-nine rabbits was cultured. The specimen of urine was obtained for culturing by catheterization as follows. After the meatus and surrounding area had been washed under a fine stream of 1 per cent lysol pumped from a syphon, a small sterile metal catheter was inserted and the urine collected in sterile tubes. It was cultured in tubes of dextrose brain broth, and on blood agar plates, on litmus lactose agar plates, or on both. The urine of forty-three of the sixty-three rabbits was sterile in all mediums used. The cultures from

TABLE II  
FORMED ELEMENTS IN URINE WHICH YIELDED A STERILE CULTURE

RABBIT	SEX	LEUKOCYTES IN EACH FIELD (low power)	PUS	OTHER URINARY FINDINGS
A-21	F	3	+	Casts
A-29	F	1 cluster of 11		
A-36		Few 5 to 6	+	
		1 cluster of 10		
A-67	F	8		One epithelial cast
A-72	M	Clusters of 25	+	
A-74	M	Many large clusters	+	
A-82		50	+	
*A-83		Clusters of 25 15	+	Few hyaline casts Few granular casts with albumin
		Clusters of from 4 to 10		
A-86	M	4 to 5	+	
		Clusters of from 3 to 10		
A-91	M	12		
A-96	M	8		

\*Necropsy revealed congestion at the corticomedullary border. Red streaks in papillae. Submucosal tissue of pelvis of right kidney, hemorrhagic.

sixteen revealed colon bacilli, in pure culture in twelve, and mixed with other organisms in four. Four of the cultures revealed streptococci or staphylococci (Table I). Eleven of the forty-three specimens of sterile urine contained pus, albumin, erythrocytes or casts (Table II).

#### SUMMARY

A colon bacilluria associated with other pathologic conditions occurs in rabbits more often than is commonly supposed. It introduces a serious source of error into experiments concerned with the production of urinary infections in rabbits, and demands extreme care in selecting a healthy stock for experimental animals and controls.

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## ETIOLOGY OF SCARLET FEVER\*

### IV. VARIATION OR TYPES OF THE ALKALI-PRODUCING ORGANISM IN SCARLET FEVER

BY R. W. PRYER, D.P.H., DETROIT, MICH.

THREE previous papers from this laboratory dealing with the bacteriology of scarlet fever have been published in this Journal. In the first two papers no mention was made of spore production, because our early work had led us to the conclusion that no spores were present. The third paper dealt entirely with the cultural and other characteristics of the organism in the spore bearing stage. We are now of the opinion that the young cultures of this spore-producing coccus-like organism are spore free and are also convinced that considerable changes in morphologic and serologic characteristics occur when the organism is carried on artificial culture media under certain conditions. But few instances of spore producing cocci are recorded in our literature and in no instance do these spore producing cocci appear to resemble the one described by us. Our later conclusions herein reported of various mutation or cyclic changes in this peculiar organism have caused us to confine our work very largely to attempts to determine something of these changes and how they may be brought about, since there is considerable direct and indirect evidence that the virus of scarlet fever varies considerably in its clinical manifestations, resistance, etc., from other organisms.

At the present time the belief is prevalent that the discharge from the throat, nose, ear, glands, etc., harbors the virus and that the majority of infections come from this source directly, although indirect infections undoubtedly also occur. In other words, the greatest factor in the dissemination of the disease is the carrier, whether he be a recently discharged case or, what all too frequently happens, a mild unrecognized case of the disease. The presence of the virus in these discharges is so generally recognized and so evident from an epidemiologic study of the disease that little more need be said.

Laboratory proof of the presence of the virus in these discharges, while somewhat contradictory, is fairly conclusive. Stickler<sup>46, 47</sup> by subcutaneous inoculation of children with material from the throat and nose of scarlet fever patients, after treatment of the material with 1 to 600 carbolic acid, apparently caused the disease. Working with monkeys, Bernhardt,<sup>3</sup> Levaditi,<sup>31</sup> Cantacuzene<sup>9</sup> and Greenbaum<sup>21</sup> reported the transmission of the disease following injection of material from scarlet fever patients. These results have been questioned by Draper<sup>15</sup> and Hektoen,<sup>24</sup> Hektoen's work being based

\*From the Laboratories of the Detroit Health Department.  
Received for publication, February 15, 1922.



on the feeding of milk contaminated by swabbings from the throat and nose of scarlet fever patients. Using pure cultures of organisms isolated from the throat of a scarlet fever patient, Cantacuzene<sup>10</sup> claims to have produced a condition in monkeys resembling scarlet fever in the human. These latter experiments of Cantacuzene are of exceedingly great interest to us, since it is possible that the organism with which he was working is the same as the one with which we are concerned. G. F. and G. H. Dick<sup>16, 17, 18</sup> working with filtrates from throat and nose mucus and washings, claim that injections of the sterile filtrate into humans fails to transmit the disease. However, they give no idea of the age of their volunteers and this we believe to be an important point, since scarlet fever is primarily a disease of childhood and adults seem to be more or less immune, irrespective of whether or not they have had scarlet fever. Working with a pleomorphic organism G. F. and G. H. Dick have obtained some suggestive results following the swabbing of throats of volunteers with suspension of this organism. It is difficult to judge from the limited amount of information published by them, but it is not at all improbable that the pleomorphic organism with which they are working is similar to the one with which we are concerned. If then these secretions or discharges harbor the virus, what is the nature of it?

One of the old ideas concerning the virus of scarlet fever is that it is extremely resistant. Consequently we believe that we are justified in saying that there is a possibility or probability of the virus being a spore producer. This idea of a resistant virus permeates our medical literature and many physicians who have had a wide experience with scarlet fever have their favorite story of this or that case caused by handling some article (toy, book, clothing, etc.), which had been used by a scarlet fever case a long time previously. It is interesting to note that comparatively few instances of a bacteriologist reporting a spore bearing organism as the cause of the disease are recorded.

One instance of a spore bearing organism present in scarlet fever is the work reported from this laboratory,<sup>45</sup> and we believe this organism to be the cause of the disease, although in this instance Koch's postulates have not as yet been fulfilled.

Since it is the purpose of this paper to show that the spore variety is the final variety, or form in the development of the organism and since this question is one of considerable concern as well as dispute among bacteriologists, it has been thought well to refer to certain papers which deal with mutation or cyclic growth among bacteria.

That scarlet fever is a very variable disease both as regards its clinical manifestations and the intensity of different outbreaks is commonly recognized. It is essentially a disease of childhood and almost 90 per cent of the cases are under the ten year age group. It is most common in winter and is almost unknown in tropical countries. Since the different forms of the disease will concern us directly, reference is made to our standard works in medicine for descriptions of these forms.

The number of so-called pleomorphic organisms which have been de-

scribed in scarlet fever is comparatively limited. Class<sup>11, 12, 13, 14</sup> made several reports concerning a pleomorphic coccus. The inclusion bodies of Döhle<sup>19, 20</sup> as reported by him and partially at least confirmed by Kolmer,<sup>28</sup> Nicoll,<sup>40, 41</sup> Nicoll and Williams,<sup>42</sup> and Bernhardt,<sup>3</sup> may possibly be considered as a variable organism, although Glomset<sup>22</sup> and others claim that they are without significance in the disease. The protozoan-like bodies described by Mallory<sup>32</sup> are apparently variable. The organism with which we are working is so extremely variable that it has seemed best to cover briefly the situation regarding variation in bacteria as brought out by different investigators.

#### VARIATION, MUTATION OR CYCLIC GROWTH OF BACTERIA

The question of variation in bacteria is one of fundamental importance and deserves much more attention than has been paid to it. It is not within the province of this paper to enter into a discussion of the terms to be used, although the writer believes that the expression pleomorphism fails to explain the results so ably described by Jones,<sup>27</sup> Mellon,<sup>33, 34, 35, 36</sup> Bergstrand,<sup>1, 2</sup> Löhnis<sup>29, 30</sup> and others. On the other hand, in the light of our present knowledge are we justified in using the expression "life cycle of bacteria?" Personally we feel that the expression "life cycle" might better be reserved for such organisms as plasmodium malaria, etc., in which part of the transformation or cycle occurs in an intermediate carrier, usually an insect or animal other than the human.

The use of the term "mutation" applied to bacteria has not been generally accepted and must be considered as indicating some unusual change in the properties of an organism.

The expression, *variety* will be used in this paper in speaking of the different forms of the organism which we believe causes scarlet fever.

The appearance of budding forms in pure cultures of bacteria have been reported by many observers. The more recent papers of Löhnis<sup>30</sup> and Bergstrand<sup>2</sup> refer to most of these reported instances and should be consulted. Wade and Manalang<sup>53</sup> report the presence of budding and branching forms in *B. influenza*. Heinemann<sup>25</sup> and others have reported observations on the assumption of coccoid forms by Parke strain No. 8 of *B. diphtheria*, while branching forms of the diphtheria organism have been reported by several workers. Hort,<sup>26</sup> using a method of single cell study devised by himself, claims that bacteria such as *B. typhosus* multiply by branching, budding and by the production of gonidial bodies in addition to the more common method of fission. This view is also held by Jones, Mellon, Bergstrand and many others. Jones working with *Azotobaacter* has observed all these reproductive forms in pure cultures. As regards *Azotobaacter* cultures, approximately the same views are held by many workers who have published their reports in recent years.

In regard to variation in serologic characteristics, considerable data is being presented all the time. Instances of change in agglutination characteristics in cultures or portions of cultures as well as in carriers of the disease are fairly numerous. We are all familiar with the fact that a freshly isolated

culture of *B. typhosus* is frequently inagglutinable until it has been transplanted many times. Benians<sup>8</sup> has apparently produced an inagglutinable Shiga dysentery by injection into rabbits of a suspension of agglutinable Shiga dysentery in tragacanth. The work of Bordet<sup>5</sup> on changes produced in *B. coli* by animal passage must also be mentioned.

To sum up then, there is considerable evidence that many of our common organisms may be varied or mutated and that to a certain extent this is brought about by influencing the method of reproduction of the organism by cultural methods or by animal passage.

#### EXPERIMENTAL

There is no doubt that when freshly isolated from the human being the organism with which we are working differs in size, morphology, and in many other ways from the end forms previously described. That it does not produce spores when living as a parasite in the body of the patient is very probable. We have tried many times to work out a system of isolation based on the assumption that spores were produced in the body of the patient, but with uniformly negative results. Variations of two fundamentally different methods have been tried.

In the first method swabs inoculated from the throat and tongue of scarlet fever patients were suspended in sterile test tubes and subjected to various temperatures for different periods. For instance, in the first stage these tubes were placed in actively boiling water for thirty minutes, the temperature of the swabs under these conditions being between 90 and 95° C. Plants made from these swabs into various media and incubated at different temperatures gave uniformly no growth. Heating at temperatures below this and varying the heating period gives unsatisfactory results.

The second method was to plant the swab directly on the various culture media and incubate these at temperatures varying from 20° to 43° C. and for different lengths of time. This growth was then washed off in sterile physiologic salt solution and subjected to sufficient heat to kill off vegetating organisms, subcultures on a suitable media being made at frequent intervals. This method also gives uniformly unsatisfactory results. This can be explained as reported in the third paper of this series, by the fact that this spore bearing coccus form, when grown in symbiosis with other organisms, fails to yield spores; also from other evidence that has been accumulated we are confident that the organism changes very considerably.

Mention was made in our third paper of a change in morphology that was noted in cultures isolated from animals dying as a result of injection of these cultures. Guinea pigs are not very susceptible to infection, but may be infected, providing the cultures are grown for some time on a fairly rich blood agar, and large doses, usually about two slants, are given. If death occurs within one day, cultures from the heart blood or peritoneal cavity usually show a large spore-bearing coccus form. From animals dying on the second to fourth day following injection, the organism isolated on autopsy is usually a peculiar small cocco-bacillus. These cultures when planted on



different sugar media are usually alkali producing. Serologically they fail to agglutinate with specific serum for the spore-bearing group, neither are they agglutinated by 16 serum. Postmortem cultures on animals dying several days after injection frequently show a hemolytic type of organism which is apparently identical with the hemolytic streptococcus of the beta type. These hemolytic organisms when carried on alkaline sugar media  $P_H$  7.8 at  $37^\circ$  C. gradually increase in size, and forms very similar morphologically to the spore-bearing forms may be seen, although no spores have been demonstrated and no alkali is produced in these cultures. This work will be continued and will be reported upon in detail in later papers.

We believe this to be of particular significance, due to the finding of Tunncliffe,<sup>48, 49, 50, 51, 52</sup> Bliss<sup>4</sup> and many others, who have practically demonstrated that the hemolytic streptococcus found in scarlet fever is different from the streptococci found in other diseases.

In our work all cultures are planted on various sugar media, and the reaction watched for a comparatively long period of time. In one instance a series of cultures which had been in the incubator for several weeks were examined microscopically and much to our surprise, we discovered one culture, No. 16, which had been isolated as a small coccus-bacillus, which produced alkali, had changed to the spore-producing, coccus-like form. This, in connection with our animal inoculation results, directed our attention into new channels, and since then much of our work has been confined almost entirely to a study of our pure cultures, in order that we might have some idea how to apply these results to the direct culturing of the patient, providing this peculiar organism, or group of organisms, has some etiologic connection with scarlet fever.

Culture No. 16 usually shows under the microscope what is apparently a mixture of cocci and bacilli. While we realize that perhaps many bacteriologists will say that this is not a pure culture, we must say that we think it is. We have plated this culture as high as ten times in succession and always without separating these forms. We might call attention also at this time to the statement made in many of our standard text books, that one method of obtaining a pure culture is by inoculating a susceptible animal and recovering the organism from the heart's blood. This culture is pathogenic for guinea pigs, and even after several successive animal passages, blood cultures show this same form, and as a matter of fact these purified cultures have been changed from this form to the big spore-bearing coccus forms. The same situation confronts us as confronted Mellon for example, in his studies on the cyclic or mutation changes in *Corynebacterium hodgkini*, etc., and which we believe he has satisfactorily answered in his later work in which single cell cultures were used. Single cell cultures have not been made by us as yet.

A point of considerable importance with regard to this culture is that when carried for some days on Löffler's media transplanting every day, forms very similar to the Klebs-Löffler bacillus are found usually predominating in the culture. These forms resemble the diphtheria organism so closely that



experienced workers may be misled by them, and call the cultures positive for diphtheria. The resemblance to Klebs-Löffler is very pronounced, and their similarity to the cultures from the discharging ears, glands, etc., following scarlet fever is a very noticeable point. This brings up the point about which bacteriologists in general are divided. Do the cultures from these discharging ears, glands, etc., contain the diphtheria bacilli or is there only a diphtheroid organism? Park inclines to the opinion that they are diphtheroids, but we cannot agree with him in all cases. Many of these discharges do, we are convinced, carry true diphtheria bacilli, but not all that are reported positive by the usual diagnostic methods are diphtheria, even though they may be reported virulent by the usual test. We would like to call attention to the advisability of making a Gram stain on these cultures, and we believe it will be admitted to be necessary. This culture, No. 16, even when

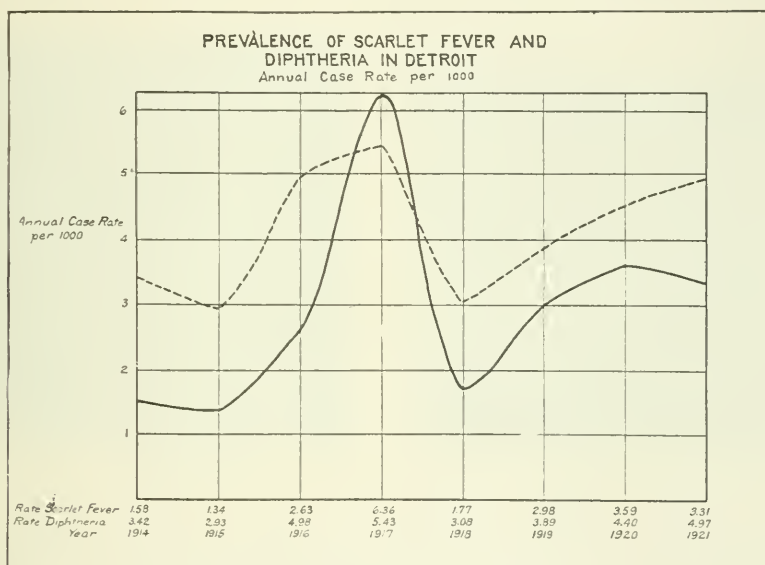


Fig. 1

very similar to the diphtheria bacillus, is frankly Gram negative. The similarity to the diphtheria bacillus is so striking, however, that it was thought advisable to see what effect, if any, ordinary diphtheria antitoxin would have on animals receiving injections of these cultures. Our animal experiment work will be reported on more in detail in a future paper in conjunction with Dr. Morgans of this staff. Certain portions of the work will be mentioned in a preliminary way here.

In this work the growth from a number of agar slants was washed off in sterile salt solution and combined so that all animals received the same dose of the same suspension. Guinea pigs of approximately 250 grams weight were used. Animals, all from the same lot, have been divided into three groups; the first, or control group, being given no antitoxin. These animals invariably died. The second group received diphtheria antitoxin at the same

time as the inoculation. Part died and part lived. The third group received antitoxin in amounts of two hundred to one thousand units, 24 hours previous to the injection of the organisms. All animals lived. This work has been repeated a great many times and with identical results. There is a possibility, of course, of there being more than one type of diphtheria bacilli. Havens has reported that he finds apparently two types of the diphtheria organisms, differing in their agglutination as well as in their toxin production. This work, however, has been vigorously disputed by Park who says that he is unable to find any marked differences in toxin production in the diphtheria organisms isolated from a large series of cases. However, we are thoroughly convinced that many times these discharges from glands and ears, etc., following scarlet fever do carry the genuine Klebs-Löffler bacilli and that diphtheria may develop in people who come in direct contact with them. In other cases, however, scarlet fever may develop.

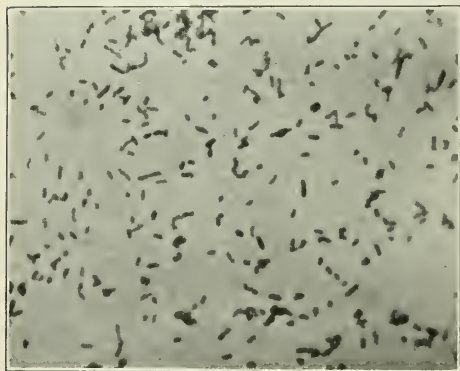


Fig. 2.—Culture No. 16; 18 hour agar culture; gram stain X1500.

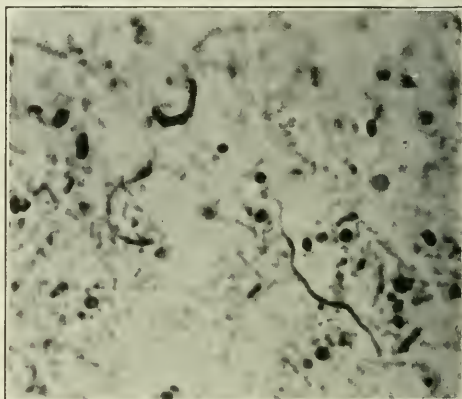


Fig. 3.—Culture No. 16; 18 day agar culture; gram stain X1500.

Cultures No. 16 when grown under conditions favorable to development of diphtheria toxin produced, after filtration through a Berkefeld filter, a poisonous substance which will kill guinea pigs. The medium that we have used in this work is similar to that described by Bunker<sup>7</sup> for use in the production of diphtheria toxin. We have succeeded in this way in obtaining filtrates which were poisonous for guinea pigs in doses as small as one-tenth of a cubic centimeter. The production of this poisonous substance appears to begin about the fifth day, and reaches its maximum between the seventh and ninth, and gradually disappears. Filtrate which has been preserved by the addition of one-fourth of one per cent tricresol and kept in the ice box at a temperature of 4 to 6° C. loses its poisonous property rather quickly. Young guinea pigs 100 to 150 grams in weight, seem to be much more susceptible. While we cannot say as yet that this represents the production of true toxin yet we believe that this opens a field of considerable importance in this work. To the person experienced in toxin work this looks like very weak toxin, but it is a matter of record that the first diphtheria toxin produced was much

weaker than this. Besson states that Roux and Yersin in their first work produced a toxin of which 30 c.c. were required to kill a guinea pig. We have noted a considerable difference in the reaction of different guinea pigs, though the weight may be approximately the same, to the inoculation of this poisonous product.

Epidemiologic studies show that in the northern portion of this country there is considerable evidence of a relationship between diphtheria and scarlet fever, and that the incidence curves of these two diseases run closely parallel. In Figure 1 the case rates of these two diseases for the city of Detroit have been plotted for the period from 1914-1921 inclusive, diphtheria being represented by the dotted line and scarlet fever by the solid line. With the single exception of the year 1917 the tendency of these curves to run parallel is very evident. During 1917 Detroit, in common with the northern part of the United States, suffered from a severe epidemic of scarlet fever. How-

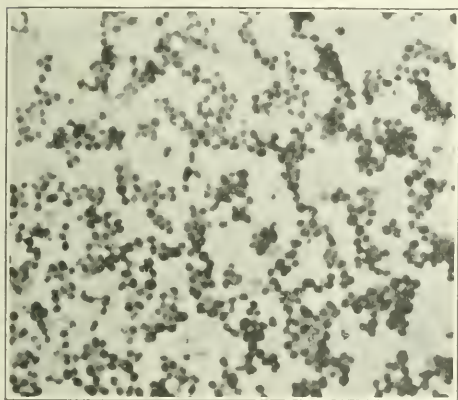


Fig. 4.—Culture No. 16. Changed to spore bearing coccus form, gram stain X1500.



Fig. 5.—Isolated from blood of guinea pig dying four days after injection of spore coccus form. Culture similar to beta haemolytic streptococcus. Culture from sucrose meat free agar six days old. Note apparent budding form. Gram stain X1500.

ever, diphtheria was very prevalent also, and although the curves cross, the general peak and valley relationship is maintained.

In view of these well recognized facts this organism which we are discussing, and which varies so widely in its morphologic and other characteristics becomes, we believe, of particular importance, and justifies the great amount of work being done on it. While this report is based very largely upon the results obtained with culture No. 16, we may say that we have a considerable series of cultures of this type which are serologically identical with culture No. 16, and which have been isolated from scarlet fever.

#### THE DIAGNOSIS OF SCARLET FEVER BY LABORATORY METHODS

Owing to the tremendous amount of routine work done in this laboratory, time for research work is at a premium; however, a limited series of cases have been examined. Thirty-five cases at the Herman Kiefer Hospital were cultured by Miss R. J. Green, in charge of the Hospital laboratory, and were

turned over to us by number for examination. In most instances two swabs were made on successive days, one from the tongue and one from the tonsils. These were cultured and examined by us, using the suggestion on which this paper is based, with the following results: Of the 35, 11 were reported as positive scarlet fever, basing our report on morphology and variation of organisms in cultures. Of these 11 reported positive, 10 were scarlet fever cases and one a patient in the tuberculosis ward. Of the 24 reported negative, 3 were scarlet fever and the others not clinically scarlet fever. This makes an average report of 77 per cent correct findings in scarlet fever and of 4½ per cent incorrect findings in other than scarlet fever. It should be stated, however, that we were unable to obtain any agglutination of the culture from the tubercular case with any of our type serum.

Very favorable results have also been obtained by examination of blood cultures both from known and unknown cases. Our method has been to use 40 c.c. of the meat free serum media (reacting  $P_H$  7.5 approximately) previously described, adding to this 10 c.c. of blood (the blood being collected either in oxalate or trypsin solution) mixing and making four or five plates, and incubating at 30° C. While the number of organisms found in ten c.c. of blood is rather low many peculiar and baffling changes in morphology are observed in subcultures, particularly when alternating between meat free sucrose media and ordinary blood agar.

The many different phases of this work are under investigation in this laboratory with the assistance of various members of the staff. It must be remembered, however, that many of the variations herein reported take place very slowly and that the conditions which govern them are very largely unknown.

The writer desires to express his appreciation to Miss R. J. Green for many favors during the course of this work and to Dr. Morgans, Miss Schueren, Miss Novy, Mr. Adams, Mr. Thomas and others of this staff for their cooperation and assistance. The microphotographs are by Miss Wagner of the Detroit College of Medicine.

#### SUMMARY

The organism previously reported from this laboratory as found only in scarlet fever shows several varieties, one of which is, under certain conditions, somewhat similar in its morphology to *B. diphtheria* and which may be a toxin producer. *Diphtheria* antitoxin protects against this culture, providing the antitoxin is given some time before the injection of the culture.

A preliminary report is made of results obtained by culturing patients sick with scarlet fever which we believe justifies the tentative conclusion that this variable organism is the cause of scarlet fever.

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# POSSIBLE DEFENSIVE FACTORS IN CANCER OF THE RECTUM\* (A STUDY OF 102 CASES)

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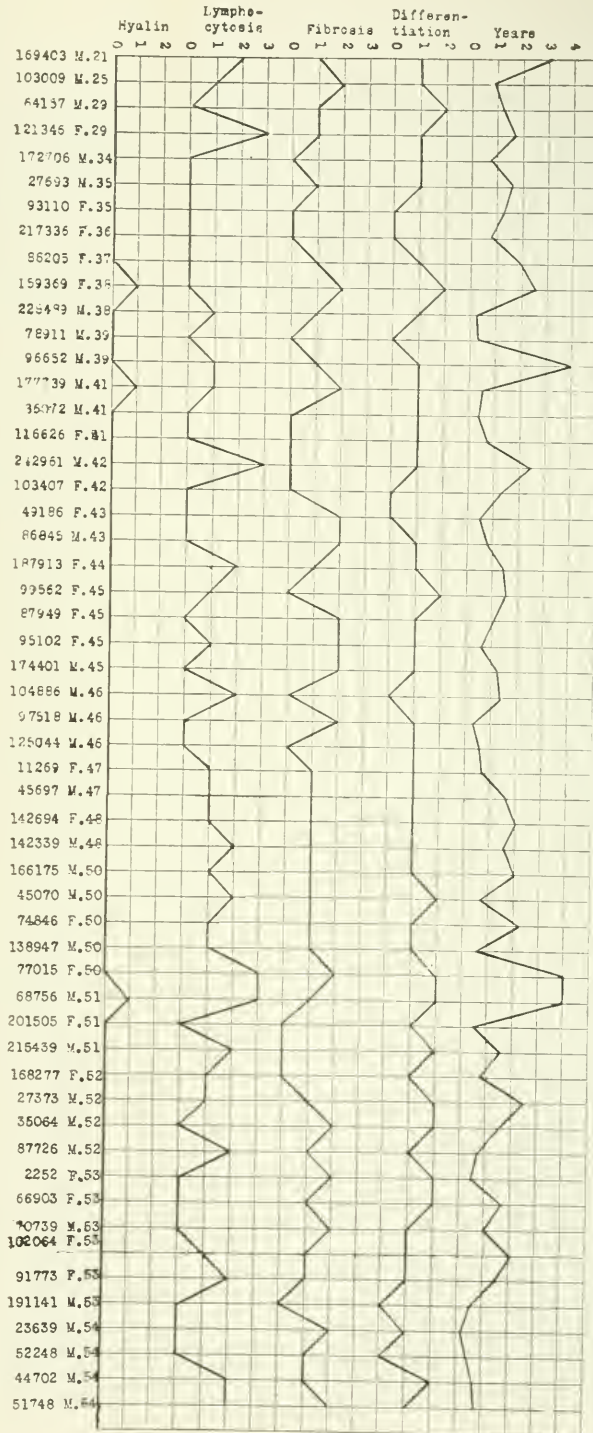
*Section on Surgical Pathology, Mayo Clinic.*

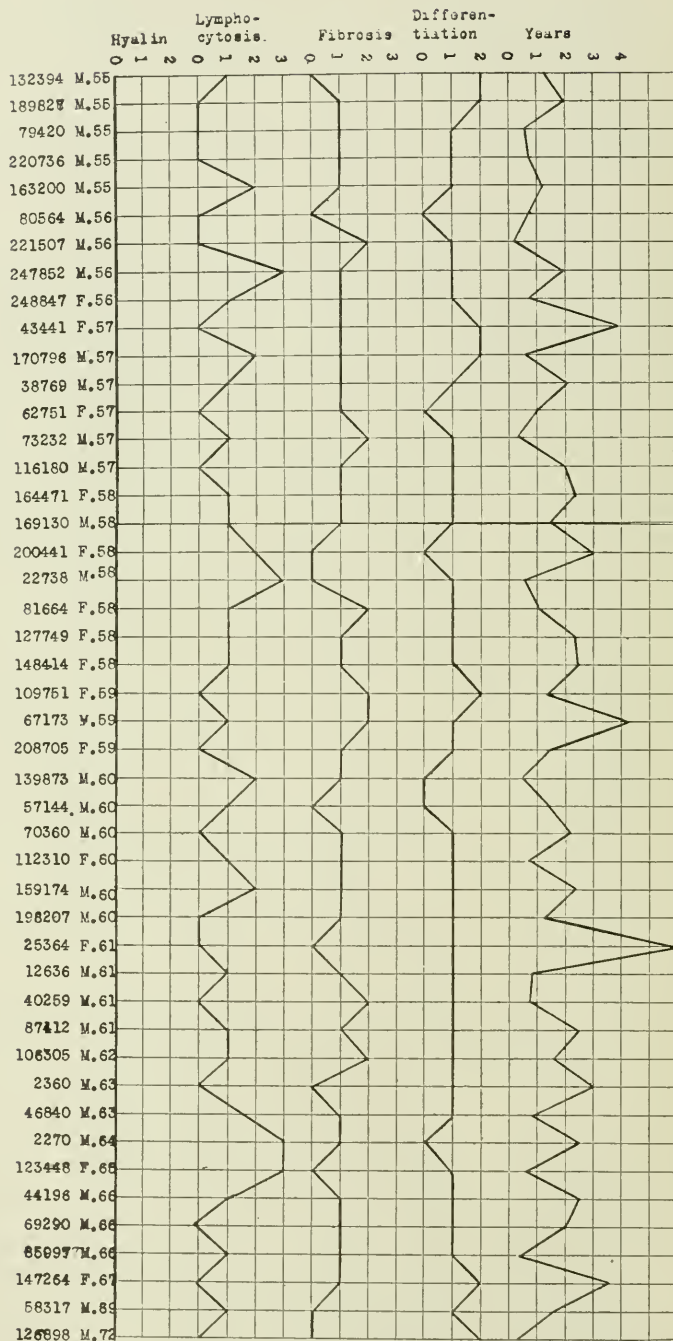
IN June, 1921, one of us (MacCarty) published a series of observations on the "Relation of cellular differentiation and lymphocytic infiltration to postoperative longevity in gastric carcinoma."<sup>1</sup> It was then stated that the presence of these factors was associated with an 82 per cent increase in average length of postoperative life. Since that observation carcinoma of the rectum has been studied in a similar manner with the exception that such possible factors as fibrosis and hyalinization were also taken into consideration. In the series, 102 cases were studied (Charts 1 to 4). As in cases of cancer of the stomach, every patient was known to have died of local or distant recurrence following radical resection.

## TABULATION OF DATA SHOWN IN CHARTS 1 TO 4.

Total number of cases . . . . .	102
Average length of postoperative life . . . . .	1.47 years
	Per cent
Frequency of differentiation . . . . .	86
Frequency of lymphocytic infiltration . . . . .	57
Frequency of fibrosis . . . . .	75
Frequency of hyalinization . . . . .	2
Frequency of differentiation and lymphocytic infiltration . . . . .	56
Frequency of differentiation and fibrosis . . . . .	70
Frequency of differentiation and hyalinization . . . . .	2
Frequency of lymphocytic infiltration and fibrosis . . . . .	47
Frequency of lymphocytic infiltration and hyalinization . . . . .	1
Frequency of fibrosis and hyalinization . . . . .	2
	Years
Average length of postoperative life with differentiation . . . . .	1.54
Average length of postoperative life without differentiation . . . . .	1.08
Average length of postoperative life with lymphocytic infiltration . . . . .	1.57
Average length of postoperative life without lymphocytic infiltration . . . . .	1.31
Average length of postoperative life with fibrosis . . . . .	1.53
Average length of postoperative life without fibrosis . . . . .	1.29
Average length of postoperative life with hyalinization . . . . .	2.33
Average length of postoperative life without hyalinization . . . . .	1.44
Average length of postoperative life with differentiation and lymphocytic infiltration . . . . .	1.59
Average length of postoperative life without differentiation and lymphocytic infiltration . . . . .	0.71
Average length of postoperative life with differentiation and fibrosis . . . . .	1.58
Average length of postoperative life without differentiation and fibrosis . . . . .	1.15
Average length of postoperative life with differentiation and hyalinization . . . . .	2.33
Average length of postoperative life without differentiation and hyalinization . . . . .	0.61
Average length of postoperative life with lymphocytic infiltration and fibrosis . . . . .	1.65
Average length of postoperative life without lymphocytic infiltration and fibrosis . . . . .	1.17

\*Received for publication, January 22, 1922.







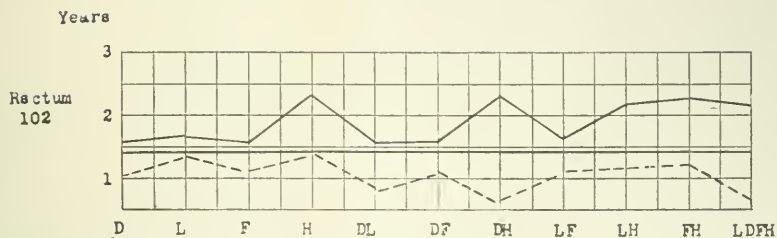


Chart 3.—Average length of postoperative life with and without factors.  
Solid line, duration of life in cases with the factor. Dotted line, duration of life in cases without the factor.

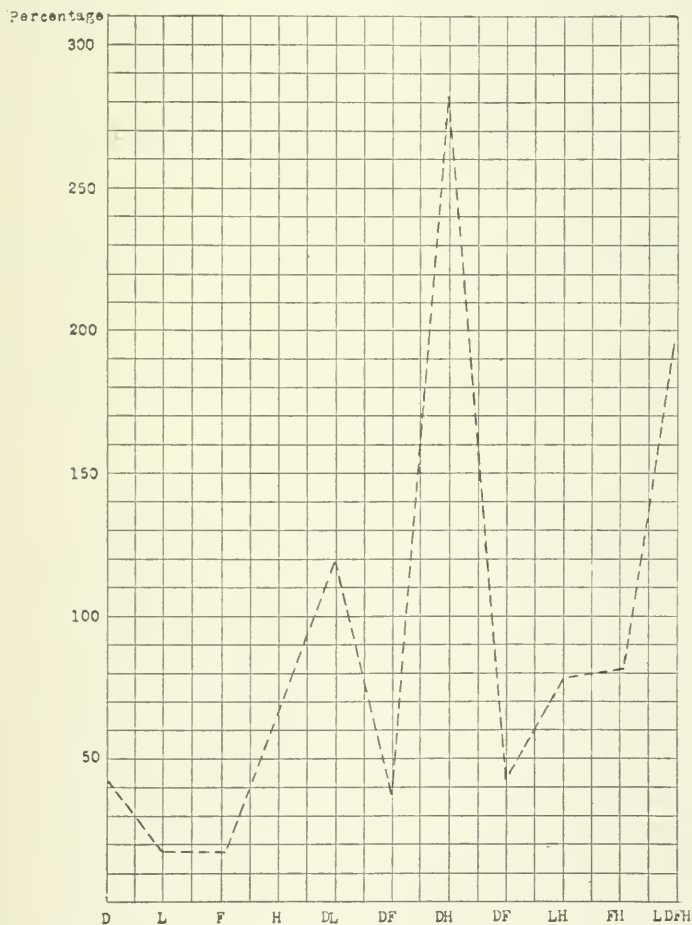


Chart 4.—Percentage increase of postoperative life with factors checked against postoperative life without factors. (Rectum.)

Average length of postoperative life with lymphocytic infiltration and hyalinization . . . . .	2.25
Average length of postoperative life without lymphocytic infiltration and hyalinization . . . . .	1.27
Average length of postoperative life with fibrosis and hyalinization . . . . .	2.33
Average length of postoperative life without fibrosis and hyalinization . . . . .	1.28
Average length of postoperative life with lymphocytic infiltration, differentiation, fibrosis, and hyalinization . . . . .	2.25
Average length of postoperative life without lymphocytic infiltration, differentiation, fibrosis, and hyalinization . . . . .	0.76

From these data certain legitimate generalizations may be made:

1. The average length of postoperative life, when the factors, lymphocytic infiltration, fibrosis, hyalinization, and differentiation, are present, is greater than when the factors are absent.

2. The average length of postoperative life, when some of the factors are present, is 40 per cent greater than the average length of life for the 102 patients, and 146 per cent greater than when none of the factors is present.

3. With all four factors present in combination the average length of postoperative life is 196 per cent greater than if none of the factors are present.

In conclusion it may be stated that from this series of observations, lymphocytic infiltration, cellular differentiation, fibrosis, and hyalinization seem to play a part in prolonging the postoperative length of life.

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## SOME SOCIOLOGICAL ASPECTS OF HEART DISEASE\*

BY DR. S. NEUHOF, NEW YORK

INCLUDING cardiovascular diseases in the term "heart disease," it is acknowledged that heart disease is widespread<sup>2</sup> and is responsible for a large proportion of deaths. Statistics vary but it is evident that valvular disease is prevalent even at the school age, and that cardiovascular disease is especially prevalent from the fortieth to the sixtieth years. The etiologic factors may be generically grouped as the infections, intoxications, and improper mode of living. This phase alone of the subject presents many aspects and angles. A few of the etiologic facts may perhaps be intelligently combated; others are unknown or are recognized only when the damage has been done to the circulatory apparatus; others have given rise to the most diverse, diametrically opposed opinions. I shall briefly sketch some of the practical difficulties of the problem, indicating some controllable, some debatable and some of the unknown causative agencies. We know for example that both lead poisoning and syphilis, especially the latter, can cause cardiovascular disease. Progress

\*Read at the November meeting of the Eastern Medical Society, N. Y. City.  
Received for publication, January 17, 1922.

has already been made through educational campaigns in schools, factories, shops, amid lead workers, etc., which have tended to decrease the ravages of these factors. This is especially true of lead but much more educational work will certainly be necessary before syphilis can be classed as a definitely controllable factor. Improper food, especially an improperly balanced, high protein diet, may have a certain influence in causing arteriosclerosis; but in spite of some experimental evidence, further intensive, exhaustive and long-continued clinical study will be required before we have positive evidence that improper feeding alone is one of the fundamental causes of cardiovascular disease. Alcoholism can undoubtedly cause cardiac disease, especially myocarditis. As already pointed out, its rôle in this respect has been exaggerated. There are hopes that alcoholism, even if not entirely eradicated, will gradually become an increasingly minor cause of heart disease. Diphtheria was undoubtedly a frequent cause of cardiac disease and death. Fortunately, toxic cases are now infrequent, and the immediate use of diphtheria antitoxin cures even some of these. Hence diphtheria has become but a small etiologic factor of heart disease. Scarlet fever may be taken as the type of a frequent infectious cause of valvular disease. We possess no specific remedy that can lessen the probability of the poison affecting the heart; our efforts are practically limited to the usual routine hygienic and symptomatic procedures which need not be discussed here. Yet, after all, the danger to the heart in scarlet fever is but a small phase of the larger problem of communicable diseases with which preventive and epidemiologic medicine must cope. Overcrowding, sanitation, education of the native-born and foreign population represent some of the sociologic aspects of the same problem.

Rheumatism, especially the acute, articular variety, chorea and tonsillitis are undoubtedly the largest and most important causes of cardiac disease in middle-aged and young. Here, likewise, we possess no specific remedy which, so far as known, will minimize the danger of endocardial involvement. It is not necessary to enter again into the extremely debatable question of the bacterial origin of the disease. Even assuming this etiology, we have no antibactericidal remedy. We possess many drugs for the control of the rheumatic articular manifestations, but for the present we can only assume that these drugs thereby lessen the liability to rheumatic endocarditis. In general, it may be stated that otherwise doubtful endocardial murmurs take on more important significance in the presence of actual rheumatism. Attention of the public should be called to the dangers of rheumatism; they should be acquainted with such simple measures as we possess to prevent rheumatism: these consist chiefly in proper clothing and protection against rain and snow in the changeable climate of the temperate zone. It may even be necessary to suggest climactic changes in order to obviate rheumatic recurrences. It is not alone the marked cases of rheumatism but also the so-called "growing pains" of children which, in educational campaigns, should be emphasized as evidences of actual rheumatism. The connection between rheumatism and the tonsils and teeth will be discussed later.

The inflamed tonsil is the next most frequent etiologic factor of endo-

carditis. About a half dozen years ago, the tonsils were eradicated upon the slightest evidence of rheumatism or of suspicion of endocarditis. The views at present are not quite as radical. Tonsillectomy has assumed much importance since the demonstration that the tonsils are often the portals of entry for endocardial infections. Tonsils which are palpably diseased or which become frequently infected should be properly and thoroughly removed if the general condition of the patient warrants it. This statement also applies to adenoids. Even here, however, a time should be chosen when the endocarditis has reached a quiescent stage, for I have seen distinct harm, and indeed recrudescence of the disease caused by untimely operation on the tonsils. However, I hold conservative views regarding the removal of apparently healthy tonsils with the idea of preventing the further spread of endocarditis. I believe that when valvular disease is already present, the removal of small and healthy looking tonsils does not tend to prevent reinfection. This view is not invalidated by the fact that tonsils which look normal in the throat show, upon removal, various pathologic changes, both macro- and microscopically. Because of their function as filtering agents and because of their constant contact with, and exposure to, bacteria, the tonsils can scarcely ever represent normal lymphoid structures. It is still a moot question whether recrudescences of endocarditis have been prevented by the routine and radical practice of tonsillectomy in all children with valvular lesions. Thus, in one excellent report, such tonsillectomies did not affect the frequency of recurrences, nor the course of the disease.

The teeth have been found to harbor, and act as foci of infection in the production of some types of muscular and joint rheumatism, especially in the adult. There have been vigorous campaigns launched both among the laity and among physicians to eradicate the foci of infection, and thus prevent rheumatism and heart disease or, when already present, to prevent their further spread. X-ray examinations of the teeth have been brought as witnesses to show how widespread dental infection may be. It may perhaps be parenthetically, yet pertinently remarked, that dentists can only indicate which teeth are diseased as well as the type of disease. It remains for the physician to determine the correlation, if any, between the dental infection and the disease in question, and to decide upon the advisability and time of removal of the dental infection. As a matter of personal observation, I believe that diseased teeth cause endocarditis in only the rarest instances. In the very few I have seen, there was dental caries and septic osteomyelitis of the jaw with general septicemia, endocarditis and death. As the result of careful and long-continued observation, I believe that endocarditis in itself does not warrant more radical or more careful dentistry than is usually required for diseased teeth and purulent foci in otherwise normal individuals. Dentistry here, as elsewhere, should follow sane lines. As with tonsillectomy, I regard routine extraction for the prevention of endocarditis as uncalled for. Extensive extractions upon the supposition that exceedingly small pus foci frequently produce endocarditis is in my opinion unwarranted by general



clinical experience and by the negative results following such practice in patients with endocarditis.

The general subject of removal of foci of infection as a prophylactic, or a curative measure in heart disease has aroused the medical mind. It is undoubtedly true that a "focus of infection" anywhere in the body, e.g., the inflamed gall bladder and oropharynx, is a potential cause of endocarditis and is hence an etiologic factor to be reckoned with. I believe, however, that its frequency as a factor has been tremendously overstated. It is one thing to diagnose a focus of infection; it is another to state that it is the cause of cardiovascular disease, and still another to state dogmatically that its removal is going to benefit the patient. As a matter of fact, it can scarcely effect a cure, for, when the diagnosis of circulatory disease is made, the pathologic damage has been done. Hence, at best, eradication of infected foci can either bring only relief or quiescence of the disease. In some older individuals with cardiorenal disease and infected teeth, I saw no effect of any kind from the wholesale extraction of teeth. In other words, prophylaxis against cardiovascular disease or the presence of the latter calls for no more radical surgical intervention and eradication of infected foci than that focus ordinarily demands. To do otherwise is to lose one's clinical balance and to practice medicine by simple rule of thumb rather than by mellowed clinical judgment.

Aside from diet and therapy, other questions regarding the management of compensated and decompensated cases of cardiovascular disease arise. Some of the commoner of these are: Shall a patient return to work? What type of work shall he follow? Shall medication be continued and, if so, how long?

With respect to these questions, no matter what the type of lesion there are two preliminary fundamental considerations to be determined, namely: The degree of compensation, and the state of quiescence of the disease. With quiescent compensated lesions, valvular or myocardial in nature, the main restriction regarding exercise should be the kind, rather than the amount, provided always it be well within the patient's cardiac reserve power. This statement requires some modification, for the type of cardiac disease plays a rôle which requires some individual discrimination. For example, patients with tremendous hypertrophic left ventricles from aortic valvular lesions are scarcely able to maintain long-continued effort without soon encroaching upon their cardiac reserve. In general, however, it may be stated that even quiescent compensated cases should avoid all exercises which call for sudden or sharp exertion, as swimming a long distance, running and tennis playing. On the other hand, golf is an excellent form of moderate exercise. It entails the necessity of being away from business and of being out in the open for a number of hours, considerations which in themselves are very desirable. In exercises as well as in work it should be emphasized that patients should keep well within their individual limits of fatigue.

The question of occupation and vocation for patients with cardiac disease has recently received wide consideration from the lay as well as from the

medical standpoints. It is gradually being recognized that many individuals with cardiovascular disease are not thereby necessarily precluded from attempting to earn a livelihood, and that, if proper work be chosen, they may become self-sustaining members of the community. Occupations and vocations at which patients sit or stand are preferable to those which require walking or stair climbing. Positions in counting houses, clerical work, draftsmanship, light manufacturing industries, working at lathes or small machinery, watchmen, are examples of the work which these patients may safely follow; but just because of these sedentary and easy occupations, exercise out in the open, chiefly walking, should be advised. It is, I believe, a therapeutic error to attempt to avoid all circulatory strain by having these patients pass an almost muscularly inert existence, for it is only by mildly stimulating the circulation by appropriate gentle exercise that the heart and circulatory apparatus are kept at their proper individual levels of efficiency. In this respect the heart does not differ from other weakened muscles whose strength is enhanced by moderate, well-planned and individualized exercise. Mild appropriate dumb-bell exercises and other calisthenics should be advised when walking is not feasible, or as additions to the latter.

It has been found that when those with cardiac disease (so-called *cardiacs*), find employment, the fact that they are handicapped and hence cannot cope with other workmen in efficiency and earning power does not depress them; on the contrary, they find happiness and satisfaction in being able to earn something which will help them toward becoming independent and self-supporting. It is, on the whole, difficult for *cardiacs* themselves to find the proper type of employment. Hence, in the larger cities, bureaus are established for the poorer and needier, through which appropriate employment may be found. Under such circumstances, the bureau head seeks information from the medical chief of the cardiac clinic and from the social service department in order to determine the functional capacity, the state and type of cardiac lesion, and the home surroundings of the individual. In this manner, some individuals have been given suitable employment and made earning members of the community. In a recent report on employment for the cardiac by I. M. Duggan,<sup>1</sup> many diversified types of employment such as watchman, telephone operators, electricians, jewelers, elevator men, seamstresses and clerks were listed.

Placement for *cardiacs* has also this important medical aspect, that in some instances it may save patients from attacks of decompensation which are due to cardiac overstrain from improper and too laborious employment. To that degree, it will decrease hospital admissions from these causes, and save hospital beds and money for other patients.

Cardiac clinics are now established in some of the larger cities, not only for group treatment of cardiac disease but also to bring home to the physicians and laity the importance and size of the problem involved. The cardiac clinic must not only be thoroughly organized from the medical standpoint, but other activities are necessary and must be organized in order to carry out important sociologic work. These will be described first, the clinic itself, last.

There is the social service department, an all-important adjuvant to the clinic. Besides routine information regarding the financial status of the patient and his family, there is correlated sociomedical work as part of its domain. If, for example, the social service head be a trained nurse, she can readily control the group exercises of the cardiac, especially of the children; her medical knowledge is sufficient for her to observe beginning dyspnea, rapid or overforceful heart action, etc., in those taking part in graded exercises and games. Follow-up work by means of which home conditions are studied, parents and relatives given proper information regarding ventilation, sanitation, clothing, food and exercise, especially stair-climbing, is also part of the work of this department. It is the most important link between the clinic and the home; without it, the clinician has no way of determining whether treatment and advice given by him can or will be carried out. If one is dealing with a child, the question of schooling and, with it, the advisability of special school classes must be considered; if with an adult, the problem of proper employment must be carefully discussed. These are but two instances of the vast number of questions upon which the work of the social department touches. When carried out in a kindly, unobtrusive way, this department tends to harmoniously bind the medical and equally important social problems of the cardiac. In large cardiac clinics,<sup>3</sup> more active educational work may be undertaken through talks given by medical and social attendants to mothers of cardiac children. These should, of course, be simple and touch upon sanitation, ventilation, proper diet, rest, exercise, the hopeful aspect of many of the cases, the importance of operative treatment of the tonsils and teeth in appropriate cases, etc.

In well equipped hospitals under whose auspices cardiac clinics are managed, there should exist helpful cooperation. Where possible, frequent consultations between cardiac and correlated departments should be the rule in order to gain proper viewpoints and well balanced judgment regarding the necessity and type of operative treatment to be carried out. Unless unavoidable because of crowded conditions, mere routine sending of patients to nose, throat, and dental clinics is inadvisable.

Where the proper hospital facilities are at hand, x-plates or fluoroscopic examination of the chest should be made in order to have data regarding the size as well as the activity of the heart. Electrocardiograms are also of value, especially in fixing the types of arrhythmias and aiding in the diagnosis of congenital lesions.

In order to obviate the necessity for the cardiac child going home for lunch or climbing stairs at school, or even visiting the cardiac clinic, special cardiac classes are established in schools, lunches are supplied, rest periods regulated, and graded exercise and games carried out. When such special classes are not available, cardiac classes can be organized in health centers or settlement houses; under such circumstances a special teacher for educational purposes and a proper nurse are required. The physician can visit and examine the children in their classrooms and give such directions and instructions as he finds necessary.



All these correlated articles are naturally predicated upon the proper formation of the cardiac clinic itself. A concerted attempt among cardiac clinics has been made to standardize and simplify the classification of cardiac disease. The following has been finally adopted:

Class I—Organic (able to carry on habitual physical activity)

Class II—Organic (able to carry on diminished physical activity)

A. Slightly diminished

B. Greatly diminished

Class III—Organic (unequal to any physical activity)

Class IV—"Possible" heart disease

(Doubtful murmurs: mainly accidental, possibly organic)

Class V—Potential (predisposing history).

One may perhaps cavil at the advisability of some of these groupings. For example, "potential" cardiacs—chiefly children that have had rheumatism, chorea and frequent tonsillar attacks—should undoubtedly be carefully examined from the cardiac point of view, but I fear that overlapping of function can scarcely be avoided if "potential cardiacs" are to be regarded as actual "cardiacs." A simple grouping of organic heart disease might perhaps be that of the quiescent, the mild and the severe insufficiency cases. But for the sake of uniformity among cardiac clinics, the first mentioned classification should be followed as adopted. Once graded, it becomes a comparatively easy task to carry out appropriate treatment. Thus Class III, the severely decompensated, are not ambulatory cases. They belong at home or in the hospital. When again beginning to compensate, the question of active and passive motion, and the time for being allowed out of bed must be decided. The first class also, those with physical signs but no symptoms, are readily disposed of. Children and adults may follow the usual conservative daily routine of normal individuals. They may practice mild sports and games; but they should eschew violent sports such as football or participation in any competitive games. Laborers should, wherever possible, choose a type of work that does not require constant extreme muscular exertion. One can only generalize by stating that all patients with organic cardiovascular disease should exercise, work or play well within their individual cardiac tolerance. I believe this warning justified in spite of the well-known fact that cardiacs are found among laborers and athletes who have never shown any circulatory symptoms. In general, those with markedly hypertrophied hearts (usually aortic lesions) should be more conservatively advised regarding the type of sports and games and such daily routine as stair- and hill-climbing. Next in the scale of conservatism are cases of mitral stenosis. One may be least conservative in those of this group (first class) which have quiescent mitral regurgitant lesion.

Class II, the mild insufficiency group, is that which usually constitutes the majority of the ambulatory patients. They likewise offer the best opportunity for improvement by means of graded games and exercises. I refer here to children alone: adults of this group, if sufficiently improved, should seek suitable employment.



Exercise among children is best achieved by graded games and drills. Games that in addition give proper posture are of special advantage. Exercises should be supervised by a doctor, trained nurse or specially instructed social worker. Various types of drills—all rhythmic—may be devised. The simplest are those in which, at the beginning, all may take part. In general, they correspond to mild setting-up exercises, leaving out sharp flexing of the abdomen. Upward and lateral movements of the arms, clapping hands above the head and in front of the body, lateral and forward, lateral and slight backward bending of the body on the trunk, bending of the knees and hips are some of the usual types of drill exercises. Military tramping and shuffling steps, marking time and other exercises will readily suggest themselves.

More active games may be devised for those who have shown no circulatory strain as a result of milder exercises. Breathlessness, prolonged tachycardia, overforceful and violent heart action, drawn and tired features are some of the more obvious, readily discernible evidences of such overstrain. Such games as hopping, pitching ball, rhythmic dancing, etc., are suggested as more active games in the better compensated cases.

An excellent adjuvant to cardiacs is some form of convalescent country home. A sojourn in the country is especially advisable for those who have recently recovered from endocarditis reinfections, or who look pale, tired and undernourished. In the latter, overfeeding should be attempted. To bring these patients to normal weight is perhaps one means of strengthening the entire organism against the inroads of infection. The home should not be too far from the city so that the patients can be occasionally visited by relatives, thus forestalling nostalgia. Convalescent homes are especially appropriate for those with fair exercise tolerance.

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## ROULEAUX FORMATION OF RED CELLS IN VARIOUS TYPES OF DISEASE\*

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### INTRODUCTION

IN immunologic work complement is usually regarded as one of the most labile constituents of blood. There are, however, two other characteristics of normal human blood which are very unstable, namely, the properties of the serum and the red corpuscles which are involved in the formation of rouleaux. Under the conditions of the following experiments, normal serum completely loses its power of producing rouleaux formation of red cells in about 24 hours at room temperature, a period of time which corresponds in a rough way to the length of time required for the inactivation of complement under similar conditions. The red cells, however, are far more sensitive and after standing for a few hours (6 to 9) at room temperature they completely lose their property of being grouped into rouleaux. In view of this striking alteration in the red cells under slight changes of environment *in vitro* it seemed desirable to determine the effect of various types of disease on rouleaux formation.

### NORMAL BLOOD

*Technic.*—In freshly shed blood of man, it has long been known that the red cells are frequently grouped into rouleaux. The physico-chemical mechanism of this arrangement of cells into rouleaux is unknown. In studying this phenomenon it is advantageous to employ a technic which permits experimental control of the concentration of cells and the quantity of serum employed. In the following experiments constant and definite results were obtained with normal blood by taking advantage of the experience of Sel-lards<sup>1</sup> who found that the heating of normal serum for a short time greatly enhances its power of producing rouleaux. Equal parts of normal serum and a 10 per cent suspension of cells in saline form practically no rouleaux. After slight heating for a short time, the serum produces intense rouleaux but this property is readily lost on slight dilution. Furthermore the concentration of cells is very important since the ease of formation of rouleaux increases in direct proportion to the concentration of the suspension of cells. This is shown in the following experiment in which equal parts of normal serum

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This paper is No. 24 of a series of studies on the physiology and pathology of the blood from the Harvard Medical School and allied hospitals, a part of the expense of which has been defrayed from a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Disease. The experimental work in connection with this work has been carried on in the Department of Tropical Medicine of the Harvard Medical School.

Received for publication, January 26, 1922.

heated for 30 minutes at 60° C. were mixed with cellular suspensions of varying concentrations.

Rouleaux formation	Strength of Cell Suspension					
	$\frac{1}{2}$ %	1%	2%	5%	10%	50%
	none	none	partial	complete	complete	complete

The following routine was adopted. A few drops of blood were collected from the finger or ear in a Wright tube and centrifugalized. The serum was pipetted off and heated in a sealed glass tube for 30 minutes at 60° C. For the red cells, a little blood was collected from the ear in 0.5 per cent sodium citrate in physiologic saline contained in a graduated 15 c.c. centrifuge tube. The cells were thrown down and washed once with saline. As a routine, a 20 per cent suspension of cells was used; any departures from this concentration are noted in the text. The precaution was taken to collect normal blood at the same time as the patient's and to test the specimens promptly after col-

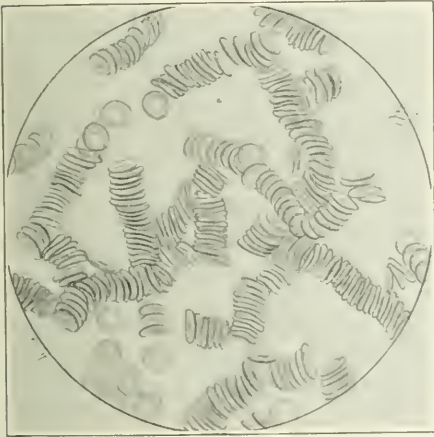


Fig. 1.—Normal cells, 20 per cent suspension, and an equal part of normal heated serum.

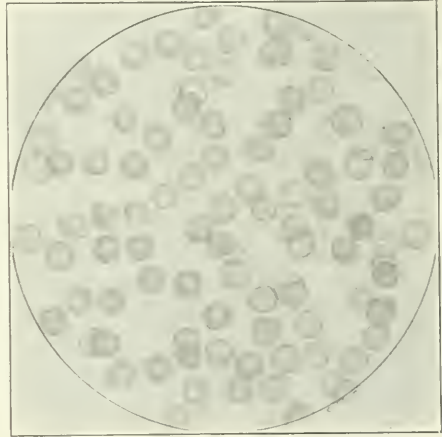


Fig. 2.—Cells from a case of Hodgkin's disease, 20 per cent suspension, and an equal part of normal heated serum. (Case No. 15.)

This figure also illustrates accurately the appearance of normal cells mixed with *unheated* normal serum.

lection on account of the rapid deterioration which occurs at room temperature. Equal parts of heated serum and cellular suspension were measured by a capillary pipette and mixed on a slide. From this mixture an ordinary hanging drop was prepared and kept at room temperature. When patients' cells were tested against normal heated serum the isoagglutination which occurred did not interfere in any way with the microscopic examination for rouleaux. Observations were made at the end of five minutes, thirty minutes, and one hour. Rouleaux formation often took place in 5 minutes and, as a rule, it did not increase after 30 minutes. An arbitrary method to describe the degree of rouleaux formation in the preparation has been used, namely, none, slight, partial, and complete: the last term indicating intense formation of cells in long chains with but few cells lying free in the field.

On each day when a specimen of patient's blood was tested a parallel

examination of normal blood was made for the purpose of controlling the technic. These controls are not always recorded in the text since they were invariably performed and always gave constantly uniform results. It will be seen that this technic is designed to detect primarily those cases in which rouleaux formation is decreased rather than increased.

*Pathologic Conditions.*—Isolated observations are found in the literature on the effect of various diseases on rouleaux formation but no systematic investigation of the subject has been conducted. These scattered observations will not be reviewed here since the technic of these observers differs so much that a comparison of results is not feasible.

*Hodgkin's Disease.*—Particular attention was given to Hodgkin's disease and the following four preparations were made in the examination of each case, viz.:

1. Heated patient's serum + patient's cells
2. Heated normal      "    +      "      "
3. Heated patient's    "    + normal    "
4. Heated normal      "    +      "      "

The patient's serum invariably behaved like normal serum showing that where rouleaux formation failed, the deficiency lay in the patient's cells. The results are shown in Table I.

TABLE I  
ROULEAUX FORMATION IN CASES OF HODGKIN'S DISEASE

SERIAL NO.	PATIENT'S CELLS	
	20%	10%
1	None	— —
(one day later)	"	— —
2	"	— —
(one day later)	"	— —
3	Partial	— —
4	Complete	Complete
5	"	Partial
6	None	None
7	Slight	None
8	None	None
(2 days later)	"	"
9	Complete	— —
10	Partial	Partial
(1 month later)	"	Slight
11	None	None
(1 month later)	"	"
12	Complete	Complete
(11 days later)	"	"
13	"	"
14	None	None
15	"	"
(2 months later)	"	"
16	Partial	Complete
17	Slight	None
*18	Complete	Complete
*19	None	None
*20	Partial	None

\*Suspected cases of Hodgkin's disease. No diagnosis made.



From this group of 17 cases in which a diagnosis of Hodgkin's had been made, there were 7 in which rouleaux formation was practically absent. These patients were under treatment with radium at the Huntington Hospital. They have not been studied sufficiently over a long enough period of time to consider the question of whether there is any relationship between the course of the disease and rouleaux formation.

*Leukemias.*—The blood of six cases of myelogenous leukemia was tested and in two, complete rouleaux formation occurred. The results are given in Table II.

TABLE II  
ROULEAUX FORMATION IN MYELOGENOUS LEUKEMIA

SERIAL NO.	PATIENT'S CELLS 20%		NORMAL CELLS 20%	
	HEATED	HEATED	HEATED	HEATED
	PATIENT'S SERUM	NORMAL SERUM	PATIENT'S SERUM	NORMAL SERUM
1	Complete	Complete	Complete	Complete
(1 day later)	"	"	"	"
2	None	None	"	"
(1 day later)	"	"	"	"
3	"	"	"	"
(2 days later)	"	Slight	"	"
4	—	None	—	"
5	—	Complete	—	"
(1 day later)	—	"	—	"
6	—	Partial	—	"

In lymphatic leukemia the results were somewhat similar to those obtained with the myelogenous type. The results are shown in Table III. These four cases of leukemia had been under treatment with radium for varying periods of time, as had the case of myelogenous leukemia and Hodgkin's disease.

TABLE III  
ROULEAUX FORMATION IN LYMPHATIC LEUKEMIA

SERIAL NO.	PATIENT CELLS 20%		NORMAL CELLS 20%	
	HEATED	HEATED	HEATED	HEATED
	PATIENT'S SERUM	NORMAL SERUM	PATIENT'S SERUM	NORMAL SERUM
1	Partial	Partial	Complete	Complete
(1 day later)	Slight	"	"	"
(2 months later)	—	Complete	—	"
(1 week later)	—	"	—	"
2	Partial	Partial	Complete	"
(1 day later)	"	"	"	"
3	"	"	"	"
(1 month later)	"	"	"	"
4	None	None	"	"
(1 day later)	Complete	Complete	"	"
(1 day later)	—	Partial	—	"

*Malignant Disease.*—In contrast to the abnormal rouleaux formation obtained in some cases of leukemia, and in Hodgkin's disease, entirely typical

rouleaux formation was obtained in eleven cases of advanced carcinomatosis and three cases of melanotic sarcoma.

*Pernicious Anemia.*—Wiltshire<sup>2</sup> makes the statement that rouleaux formation is defective in pernicious anemia. Five cases of typical pernicious anemia were tested and all gave entirely normal results.

*Nephritis.*—Five cases of advanced chronic nephritis were tested and in each instance normal results were obtained both with the patient's serum and with the patient's cells.

*Infectious Diseases.*—No abnormal results were obtained in any of the twenty-one cases of infectious diseases which were tested during the acute stage. The following list comprises the cases which were examined: Ten of pulmonary tuberculosis, 4 of lobar pneumonia, 3 of diphtheria, 2 of measles, one of whooping cough, and one of ulcerative endocarditis.

#### SUMMARY

In the study of rouleaux formation in disease, emphasis is placed upon the necessity of a technic which permits quantitative measurements by varying the dilution of the serum and the cellular suspensions.

In 7 out of 17 cases of Hodgkin's disease rouleaux formation was completely lacking under conditions in which normal red cells formed intense rouleaux. In all the cases of leukemia examined, rouleaux formation was lacking to a greater or lesser degree.

It was thought that this deficiency of the red cells from cases of Hodgkin's disease and leukemia might be due to the anemia which occurs in these diseases and that the change is not due to the anemia *per se*, is shown by the fact that the red cells of pernicious anemia formed rouleaux well.

Normal rouleaux formation always occurred in the cases of cancer and sarcoma and in the cases of infectious disease that were tested. These results were clear cut. On the contrary the results obtained in cases of leukemia and Hodgkin's disease were variable. It would seem that if there is any valuable information to be gained by testing for rouleaux formation, some finer development or adjustment of technic than that used must be established.

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## LABORATORY METHODS

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### THE PRECIPITIN TEST IN THE DETECTION OF BACTERIUM DIPHtheriae\*

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G. H. SMITH, PH.D., AND C. E. KAUFMAN, M.D., NEW HAVEN, CONN.

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VARIOUS modifications in the routine method for the bacteriologic diagnosis of diphtheria have been suggested from time to time. In all, the underlying purpose has been to reduce the time necessary for making the laboratory diagnosis, either by the utilization of a culture medium better adapted to the growth of the organism or by the application of a staining procedure which will more certainly detect *Bact. diphtheriae* when present in limited numbers or in atypical form. None of these methods have replaced the older technic since the difficulties inherent in bacteriologic diagnosis could not be overcome entirely by any of the modifications suggested.

It seemed possible that the period necessary for making a diagnosis might be shortened by resorting to serologic methods. Furthermore, if the serologic test possessed specificity the difficult matter of differentiating between *Bact. diphtheriae* and the pseudodiphtheria forms, particularly in young growths, might also be avoided. Earlier studies in pneumonia<sup>1, 2</sup> and more recent work in gonorrhea<sup>3</sup> have shown that fairly satisfactory and expeditious results can be secured by the precipitin test. An attempt has been made to adapt this test to the detection of *Bact. diphtheriae*.

The value of such a method depends upon the specificity of the reaction as well as upon the delicacy of the test. By means of agglutination and agglutinin absorption tests Langer,<sup>4</sup> Przewoski,<sup>5</sup> Lipstein,<sup>6</sup> van Riemsdijk,<sup>7</sup> Mason,<sup>8</sup> and others have shown that antibacterial diphtheritic sera react with diphtheria antigens in a specific manner and show no cross reactions with pseudodiphtheria strains. The work of Durand<sup>9, 10</sup> would indicate that a grouping of diphtheria strains into antigenic groups occurs when monovalent sera are employed in dilutions above the nonspecific zone. It thus appeared that any reactions secured might possess the requisite degree of specificity.

The technic for preparing the reagents;—precipitin and precipitinogen—the procedure for conducting the test; and the results which have thus far been secured are described below.

The precipitating sera have been derived from two sources, (a) rabbits immunized with single strains of *Bact. diphtheriae* and (b) antidiphtheritic

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Received for publication, November 16, 1921.

horse serum.\* Rabbits were immunized with freshly isolated strains from clinical cases of diphtheria and the organisms used were of established virulence and presented all the characteristic cultural requirements of *Bact. diphtheriae*. In addition, for purposes of controlling the tests, immune sera were produced with diphtheroid organisms. Not all rabbits so treated have yielded sera of suitable precipitating properties, yet with certain animals sera of high titre have been secured. The difficulties associated with the production of such sera led us to try an antidiphtheritic horse serum and the results secured with this serum were sufficiently favorable to justify us in discontinuing the immunization of rabbits.

For the preparation of antigen, extracts of *Bact. diphtheriae* were secured by breaking down the organism with an alkali hypochlorite solution. By this method antigens have been prepared not only from pure cultures of *Bact. diphtheriae* but also from mixed cultures and from nose and throat swabs taken directly from the patient. Following the observation that satisfactory antigens could be derived from swabs, these have been employed exclusively since at least twelve hours can be saved by eliminating cultural procedure. The method of extracting the antigen is essentially that of Krumwiede and Noble. It consists in introducing the swab into 0.5 to 1.0 c.c. of "Antiformin" and extracting by boiling for several minutes. The solution is cooled and neutralized in large part before the addition of indicator. This neutralization is accomplished by the addition of 3 to 5 drops of N/4 HCl, after which indicator (phenolphthalein) is added and the neutralization quickly completed by further addition of the requisite amount of acid. The end-point is approximately  $P_H$  8.3. This solution is precipitated by the addition of 5 to 10 volumes of 95 per cent alcohol, after which the precipitate, which appears immediately or after standing for a short time, is collected by centrifugation. Precipitation may be hastened and the volume increased by heating immediately after the addition of the alcohol. The precipitate is taken up, with boiling, in 1 to 2 c.c. of physiologic saline. Usually such extracts are practically water-clear; if not, centrifugation will remove the turbidity. Occasionally an extract will again become alkaline upon the addition of the salt solution or during final heating, and under such circumstances the reaction should be restored with acid. The antigens should be prepared in as concentrated a form as possible; in some instances where questionable reactions have been secured a concentration of precipitinogen has been effected by further boiling.

It may be added that swabs secured from the nose and throat have remained at room temperature for as long as 60 days and have still been found suited to the extraction of antigen, although the yield or activity of the antigen so obtained has appeared to be diminished.

The test itself consists simply in layering these extracts upon the precipitating sera. In order to conserve sera and to conduct the reaction with a minimal amount of concentrated antigen the tests are set up in small tubes of about 4 mm. diameter. As a rule the reaction appears almost immediately

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\*Kindly supplied by the H. K. Mulford Company.



in the form of a ring of precipitate at the point of contact of the two liquids. With the weaker antigens incubation at 37° C. for 10 to 30 minutes may facilitate precipitation.

The reactions with the antidiphtheritic serum are always controlled by combination of the antigen with a normal serum and with a diphtheroid immune serum. The entire procedure—from the time of taking the swab from the patient to reading the results in the laboratory—can all be accomplished well within one hour. When a number of tests are to be made the procedure can be carried through on the entire series simultaneously with a considerable saving in time.

With this technic a considerable number of swabs have been tested and the results of a series of such examinations are presented in the following table:

TABLE I

CULTURAL EXAMINATION		SEROLOGIC EXAMINATION	
	Positive	Positive	Per cent
	44	43	97.7
		Negative	
		1	2.3
	Negative	Positive	
	30	8	26.6
		Negative	
		22	73.4

From this table it is clear that the method outlined above will yield satisfactory results if cultural examination is accepted as the standard for comparison. When *Bact. diphtheriae* was present on the swab in sufficient numbers to yield a positive culture the precipitin test was positive in 97.7 per cent of the cases. With specimens culturally negative the precipitin test agreed in 73.4 per cent and the discrepancy between the two methods is in the direction of an excess of positive findings with the serologic method. It may be said, however, that none of these "negative culture-positive precipitin" reactions occurred in cases in which there was any reason to suspect diphtheritic infection. Moreover in several instances in which cultures and precipitin tests were made upon individual cases over a series of days it developed that although cultural examinations did not yield uniformly consistent results from day to day the precipitin tests were positive throughout the period.

The excess of positive precipitin reactions in the series giving negative cultural findings suggests that organisms may be detected by the serologic method even though they are present in numbers insufficient to give a positive growth upon media when inoculated in mixed culture. The difficulties connected with securing positive cultures when but few organisms are present have been pointed out by Wang<sup>11</sup> and others.

A certain number of questionable reactions may be expected. In the series upon which Table I is based one autolysate was secured which gave a precipitate with normal serum and none with an antidiphtheritic serum; and

in the case of a second autolysate positive reactions were secured with both the normal and immune sera. In this last instance dilution of the antigen caused the reaction with the normal serum to disappear while that with the antidiphtheritic serum persisted. The greatest possibilities for error in performing the test are associated with a too great acidity or alkalinity of the precipitinogen, or in failing to drive off all alcohol during the final heating. In no instance have we observed any nonspecific effects due to the presence of other organisms in association with *Bact. diphtheriae* upon the swabs, nor have we noted any quantitative differences that would suggest an error in the method such as might be associated with the antigenic grouping described by Durand.

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## A MICROMETHOD FOR NONPROTEIN NITROGEN USING TWO-TENTHS OF A CUBIC CENTIMETER OF BLOOD\*

BY C. M. WILHELMJ,† St. Louis, Mo.

A METHOD for the estimation of nonprotein nitrogen in which the blood can be obtained from the finger or lobe of the ear, obviously has its place in clinical routine. It is becoming more and more important for the clinician to determine at frequent intervals the effect of treatment, diet, and so forth, in the various conditions complicated by a high nonprotein nitrogen content of the blood. The method described here gives results which I believe are sufficiently accurate for clinical interpretation and the ease with which the blood is obtained makes it possible to have determinations done at short intervals without great inconvenience to the patient.

## THE METHOD

The blood is obtained from the lobe of the ear or the finger, and for this purpose a spring lancet is especially suitable since the depth of the puncture can then be accurately gauged and sufficient blood obtained by one

\*Received for publication, October 28, 1921.

†From the Medical Service of the Jewish Hospital, St. Louis, Mo.

puncture. The blood is drawn into the blood pipette up to the .2 c.c. mark and immediately discharged into the tube graduated at 2.5 c.c., which contains a very small amount of finely powdered potassium oxalate. The blood pipette is then rinsed *twice* by drawing distilled water up to the .2 c.c. mark and this is added to the blood. Five per cent trichloroacetic acid is then added up to the 2.5 c.c. mark, a few drops at a time, shaking after each addition. The coagulum is a chocolate brown color. The mixture should next be stoppered and allowed to stand for 20 or 30 minutes. At the end of this time it is filtered, using a filter paper just large enough to accommodate the whole mixture. One c.c. of the protein-free filtrate is transferred to the digestion tube,\* and .2 c.c. of the digestion mixture added. The digestion is preferably done over a microburner, the tube being held in a test tube clamp or a band of heavy paper. The contents of the tube must be kept in constant circular motion all during the digestion in order to prevent loss of fluid by "bumping." The fluid rapidly boils down until only a small amount remains and this gradually becomes dark brown in color. Dense white fumes soon begin to fill the tube and when this occurs the rate of boiling should be slightly reduced. The color of the fluid gradually becomes lighter and finally becomes clear pale green; the boiling should be continued for about two minutes after this change occurs. Complete digestion should not take over 12 or 15 minutes. The solution is now cooled, and distilled water added, and the contents transferred to one of the *ungraduated* tubes of the aeration apparatus. The digestion tube is repeatedly rinsed with distilled water and these rinsings added to the contents of the aeration tube until it has been made up to a volume of about 8 or 10 c.c. (not over). One-half c.c. of N 10 Hydrochloric acid is put in the *graduated* aeration tube and the volume made up to 5 c.c. with distilled water. About 5 c.c. of fairly strong sulphuric acid is put in the remaining *ungraduated* tube and the apparatus connected with the filter pump as shown in the figure. Just before the air current is started, two pieces of stick sodium hydroxide about  $\frac{1}{4}$  of an inch long are cut in half and added to the middle tube which contains the digested filtrate. The air current is then started and run at slow speed for the first five minutes and rapidly for 20 or 25 minutes longer, depending upon the rate of the air current used.\* When the aeration is complete, 1 c.c. of the Nessler-Winkler solution is diluted with an equal volume of water, and 1 c.c. of this diluted solution added to the contents of the graduated aeration tube, and the volume made up to the 8 c.c. mark with distilled water. The resulting color is a reddish brown but should be absolutely crystal-clear since even a faint turbidity makes it unfit for comparison. The Nesslerized solution is now thoroughly mixed and transferred to the cup of either a Kober or Boek-Benedict colorimeter and compared with a standard solution. I have used

\*The digestion tube may be lightly graduated at 1 c.c. and the filtrate run directly from the funnel into this.

\*With a sufficiently rapid air current this time may be cut down to 10 minutes. The length of time required with a given apparatus may be determined by adding Nessler solution to the middle tube, which should remain colorless if all of the ammonia has been driven off.

both of these colorimeters with this method and find the 8 c.c. of solution to be sufficient to make the comparison.

The standard is prepared by adding 1.5 c.c. of standard nitrogen solution to 75 c.c. of distilled water, in a 100 c.c. graduate; to this 5 c.c. of undiluted Nessler-Winkler solution is added, and the contents made up to 100 c.c. with distilled water. The comparison is usually best made with the standard cup set at 20.

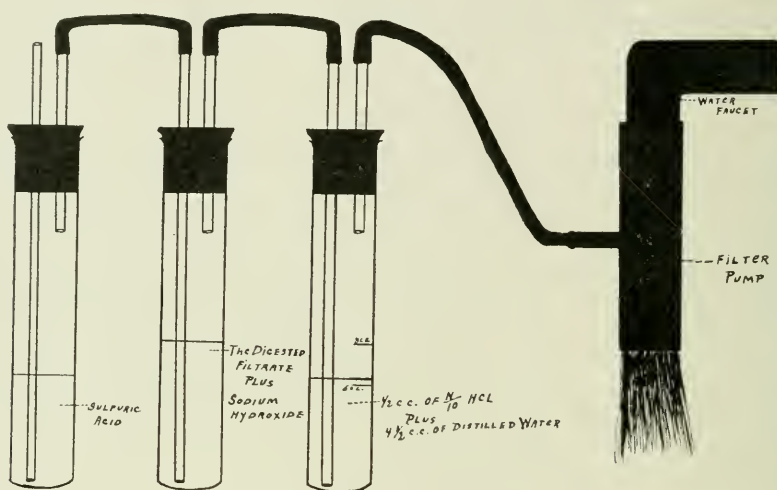


Fig. 1.

#### Calculation:

Since the equivalent of .08 c.c. of blood is compared with the standard at a volume of 8 c.c. the equation becomes:

$$\frac{\text{Reading of the standard}}{\text{Reading of the unknown}} \times \frac{30}{1} = \text{mg. of nitrogen per 100 c.c. of blood.}$$

#### Example:

Standard 20

Unknown 15

$$\frac{20}{15} \times \frac{30}{1} = 40 \text{ mg.}$$

#### Reagents Required\*

1. A 5 per cent solution of trichloroacetic acid.
2. Digestion mixture consisting of: 1.5 c.c. of 10 per cent copper sulphate solution; 15 c.c. of distilled water; 15 c.c. of concentrated sulphuric acid; 1.5 grams of potassium sulphate. This mixture is thoroughly shaken and kept in a well-stoppered bottle.
3. Stick sodium hydroxide.
4. N/10 hydrochloric acid.
5. Nessler-Winkler solution†

Mercuric iodide .....	10 gm.
Potassium iodide .....	5 gm.
Sodium hydroxide .....	20 gm.
Water .....	100 c.c.

\*Great care should be taken to secure nitrogen-free reagents.

†Directions from Hawk's Physiological Chemistry.



The mercuric iodide is rubbed up in a small porcelain mortar with water, and washed into a flask and the potassium iodide added. The sodium hydroxide is then dissolved in the remaining water and the cooled solution added to the above mixture. The solution cleared by standing is preserved in a dark bottle.

#### 6. Standard nitrogen solution.

Prepared by dissolving 0.764 grams of pure ammonium chloride in 1000 c.c. of distilled water. One c.c. contains .2 of a mg. of nitrogen.

#### 7. Powdered potassium oxalate.

#### *Apparatus Required:*

1. A blood pipette graduated at .2 c.c. These can be obtained from Eimer & Amend, New York, who supply a similar pipette with the Epstein Microsaccharimeter.

2. A small short tube accurately graduated at 2.5 c.c.

3. A small, long-stemmed funnel.

4. A digestion tube of thin hard glass, preferably about 1.1 cm. in diameter and not over 9.5 cm. in length.

5. Aeration apparatus consisting of: A filter pump; 3 test tubes, 1.8 cm. in diameter and 15.5 cm. in length, one of the tubes to be accurately graduated at 5 and 8 c.c. Three two-hole rubber stoppers to fit the above tubes, with holes to fit the glass tubing. Glass tubing about 3 mm. in diameter. Rubber tubing to connect the glass tubing. The aeration apparatus is connected as shown in Fig. 1.

TABLE I

CASE	MICROMETHOD	FOLIN METHOD	DIFFERENCE
1 Cardio-Nephritic	(1) 41.4 (2) 40	40	+1.4
2 Acidosis?	(1) 42.1 (2) 45.4	40	+2.1 +5.4
3 Healthy	(1) 30 (2) 35	35.1	-5.1 - .1
4 Skin condition	38.6	38.2	+ .4
5 Septic pneumonia	41.3	43.1	-1.8
6 Inguinal adenitis	(1) 40 (2) 40	34.5	+5.5 +5.5
7 Luetic stricture of rectum	40	37.5	+2.5
8 Abortion	(1) 33.3 (2) 33.8	31.6	+1.7 +2.2
9 Acute nephritis	35.2	32.4	+2.8
10 Uremia	69.2	66.4	+2.8
11 Healthy	38.7	33.6	+5.1
12 Nephritis	40	38.7	+1.3
13	42.8	41.4	+1.4
14 Healthy	(1) 29.6 (2) 30	—	—

## DISCUSSION

The method of Folin<sup>1</sup> is without doubt the most accurate colorimetric procedure for the estimation of nonprotein nitrogen and hence was selected as a standard of comparison for this method. Table I shows the comparative results, with duplicate determination by the micromethod in some cases. Here it is seen that in a total of 16 readings the micromethod gave higher results than the Folin in 13 and lower results in 3. The greatest difference is plus 5.5 mg.

Greenwald<sup>2</sup> who first advocated the use of trichloroacetic acid as a protein precipitant, advised that the protein-free filtrate be shaken with nitrogen-free kaolin and again filtered. He stated that when this step was omitted the results were about 2 mg. higher than when it was used, and since this step has been omitted in the above method this, no doubt, accounts for the almost uniformly higher readings. However, a difference of 5 mg. is hardly sufficient in clinical interpretation to lessen the usefulness of this method. This method is intended for clinical diagnostic purposes, not for research, where a higher degree of accuracy is desirable.

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## THE CELLULAR ELEMENTS OF THE CEREBROSPINAL FLUID\*

BY A. LEVINSON, M.D., CHICAGO, ILL.

THE cellular elements of the cerebrospinal fluid occupy an important place among the many unsettled problems of this body fluid. The origin of the cells in the normal fluid is no more settled than is the origin of the fluid itself. Nor are the types of abnormal cells in various diseases a matter of common knowledge. Yet a close study of cerebrospinal fluid does show certain types of cells in certain diseases of the meninges or of the brain. Furthermore, the cells may be used as an aid in diagnosis, and occasionally also as a guide in treatment. It is to the various types of cells in the fluid that I would like to call attention here.

## TECHNIC

Before discussing the different types of cells, I would like to call attention to the fact that not every stain used for blood cells brings out the cells in the cerebrospinal fluid vividly. Wright stain, for instance, does not bring out the structure of the cells in detail. Nor does methyl-violet or carbolthionin. In my work I found methyl-blue the best stain for the cellu-

\*Received for publication, November 5, 1921.

lar structures of the cerebrospinal fluid. Next to methyl-blue, I found the Gram stain which is counterstained with methyl-blue most useful. Safranin makes an excellent counterstain from the standpoint of the bacteria. It does not, however, always bring out the details of the cells. The same is true with Bismark brown. Gram stain which is counterstained with methyl-blue would answer both purposes, in showing the type of bacteria and the cells. Some workers, however, cannot distinguish between violet and blue. It is therefore best to use the Gram-safranin stain for the bacteria and plain methyl-blue for the cells. Both stains can be made in a few minutes as against staining with Wright stain which is time consuming.

Another important point in the technic is to examine the cells in the fluid soon after the fluid has been withdrawn from the body, otherwise the cells degenerate.

#### NORMAL FLUID

It is agreed upon that normal fluid contains only four to six cells per c.m. and that all of them are small lymphocytes. My belief is that the cells in normal fluid are derived from the blood, the chorioid barrier permitting only a minimum number of cells to pass through its walls, just as it permits small amounts of the chemical constituents of the blood to pass through into the fluid. This, however, is merely an opinion. The origin of the cells in normal fluid has not been settled as yet.

#### PATHOLOGIC FLUID

Any infection of the meninges gives rise to an increased number of cells in the cerebrospinal fluid. Irritation of the meninges or of the brain may also cause an increase of cells. The number of cells, however, is not nearly as great in irritative conditions as in destructive processes. In meningism for instance, although the amount of fluid is increased the cells may not be increased in number, and when they are increased, they are all lymphocytes in type. In tuberculous meningitis and in certain stages of poliomyelitis, the cells are mainly lymphocytes. On the other hand, the cells in all suppurative meningitides are polymorphonuclear in type. In addition to the polymorphonuclear cells, however, there are also many endothelial cells. This type of cell is typically predominant in meningococcus meningitis. The same is true with the phagocytes which are present in large numbers in meningococcus meningitis (Figs. 1 and 2). In tumor of the brain, large lymphocytes are often present.

A description of the cells found in cerebrospinal fluid is given below in order of their frequency, based on my own work and on the work of other authors.

1. *Small lymphocyte (small mononuclear leucocyte).*—This type of cell, which is present in small numbers (1 to 6) in normal fluid and which is present in large numbers in tuberculous meningitis, poliomyelitis and luetic infections of the meninges, is the size of a red cell and contains a deeply staining nucleus, which fills nearly the whole cell. The small amount of remaining protoplasm takes the Wright, Unna-Pappenheim, or methyl-blue stain less deeply than the nucleus and contains no granules.

2. *Large lymphocyte (large mononuclear leucocyte).*—This cell which is occasionally present in normal fluid is present in large numbers in chronic inflammatory conditions of the meninges such as luetic affections of the meninges and in brain tumor. The cell is twice the size of a red cell and contains a round, less frequently an oval, nucleus which fills the large part of the cell and which stains deeply.

3. *Polymorphonuclear leucocyte.*—This type of cell is found in great numbers in the fluid of all septic processes of the meninges, in meningeal hemorrhage and at times also in brain abscess. The cell is one and a half to two times the size of a red blood cell. The nucleus is subdivided into 2, 3, or 4

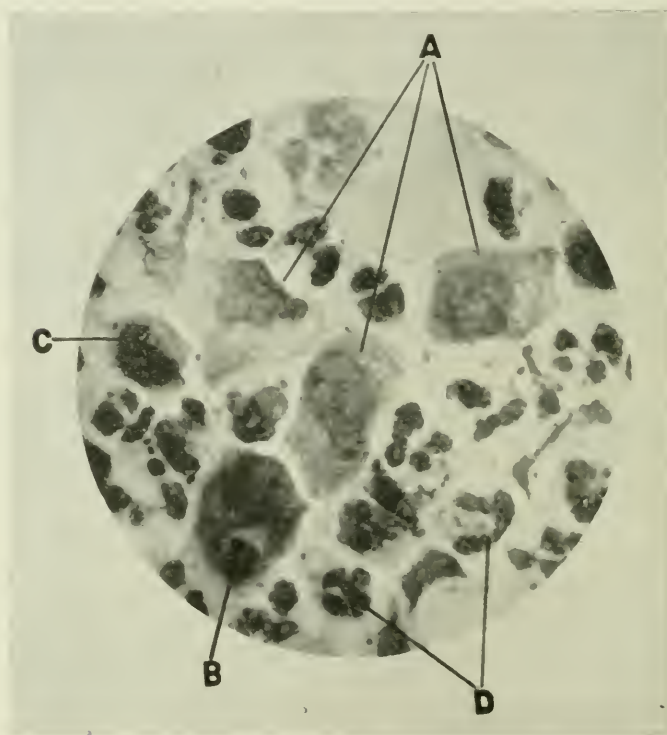


Fig. 1.—Photomicrograph showing types of cells in pathologic cerebrospinal fluid. X1000. A, endothelial cells. B, endothelial cell which has engulfed a polymorphonuclear leucocyte. C, large mononuclear cell. D, polymorphonuclear leucocytes.

lobules and the protoplasm contains many granules, the granules being either small and neutrophilic in character or large and eosinophilic. Most of the polymorphonuclear leucocytes found in the fluid are neutrophilic, eosinophilic leucocytes being found only seldom.

4. *Endothelial cell. (Endothelial leucocyte, transitional cell.)*—This type of cell is supposed to be derived from the endothelial lining of the meninges. It is present in large numbers in the fluid of acute meningitis, especially in the fluid of meningococcus meningitis, and to a lesser degree in the fluid of subacute inflammatory processes of the meninges.



The endothelial cell is larger in size than a large lymphocyte. It contains a small, round, or oval, eccentrically placed nucleus, and a large amount of weakly staining protoplasm. The protoplasm often looks transparent and in places even seems to contain vacuoles.

Under the heading of endothelial leucocyte may be included the transitional cell, which is a large cell with indented or horse-shoe shaped nucleus. Many authors consider the transitional cell as an endothelial leucocyte.

The Gitterzellen of the German authors is an endothelial cell in which the protoplasm is subdivided by fissures.

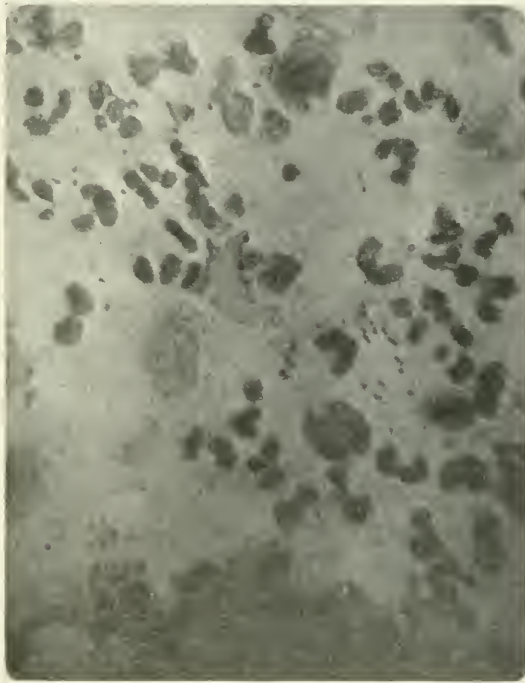


Fig. 2.—Direct smear of cerebrospinal fluid from another case of meningococcus meningitis.

5. *Phagocyte*.—In meningococcus meningitis and less frequently in other meningitides large cells containing smaller cells inside are seen in the fluid. The presence of phagocytes is not always an indication of the phagocytic action on the cells and has no prognostic significance.

6. *Fibroblast*.—This cell which is derived from connective tissue is present in chronic inflammations of the meninges. The cell body is elongated, pointed at both ends and contains a small round or oval nucleus.

7. *Plasma Cell*.—This cell which is found in old standing processes such as general paresis, is the size of a large lymphocyte or a little larger. It contains a granule and a deep-staining nucleus placed eccentrically.

8. *Erythrocyte*.—In fluid from meningeal hemorrhage, in all acute inflammatory processes and whenever blood has been obtained by an unsuccessful spinal puncture red blood cells are found in the fluid.

9. *Tumor and cyst cells.*—Sarcomatous cells may appear in the fluid of a case of sarcoma of the meninges. Echinococci have been found in the fluid from cases of echinococcus cysts. Sicard reported a case where actinomyotic granules were found in the fluid.

10. *Unclassified cells.*—In all destructive processes of the meninges, cells are met with in the cerebrospinal fluid in all stages of degeneration. The cells vary in size and shape and simply indicate destruction of the meninges.

# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, JULY, 1922

No. 10

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## EDITORIALS

### *Further Studies on Pellagra*

THE World War gave frequent opportunity for the study of pellagra, especially in Egypt and Asia Minor. In the fall of 1915 some 4,000 Armenians, men, women and children, who had been driven from their homes by the Turks and who had been wandering under great hardships in the mountainous regions of Syria, were collected by the British and carried to Port Said. They spent the winter of 1915-1916 in this camp on a restricted diet in which the gross protein per man per day amounted to 64 grams, while the available protein was only 51.5 grams and the biologic value of the protein only 23. By the spring of 1916 ten per cent of these refugees had developed pellagra. They were immediately supplied with an antipellagrous ration furnishing 99.3 grams of gross protein, 83 grams of available protein and 59.15 as the biologic value of the protein. On this diet they rapidly recovered.

Wilson<sup>1</sup> has gone quite minutely into the dietaries upon which people have developed pellagra, as well as upon antipellagrous rations, and has quite convinced himself that pellagra is not due to the absence from the food of a specific vitamin. He is equally convinced that this disease is not due to a deficiency in fat or to improper fats in the food. He points to the fact that the rise in the price of foodstuffs has restricted the supply of proteins much more than it has that of either carbohydrates or fats. He says: "That a deficiency in the amount and quality of the food is of primary importance in producing the disease, has been pointed out by the majority of observers from Casal onwards; also the reciprocal fact that a generous diet will cure the disease." All the pellagrous diets are deficient in proteins, and this is quite independent of whether or not they contain maize or maize products. In a few instances, while the ration has contained enough protein, the protein has been of inferior biologic value or it has failed of digestion and absorption.

Wilson concludes his rather exhaustive study of the dietary of this disease as follows: "(1) Pellagra is the ultimate result of a deficient supply of protein. (2) The sufficiency or insufficiency must be judged of by the biologic value of the protein, estimated on the amount available for assimilation and not on the gross protein; 40 may be taken as the minimum safe value for this factor. Below this, cases are likely to occur in the affected community; owing however to the great normal variations in the minimum protein requirement, many individuals, the biologic value of whose daily protein intake is as low as 20, will escape the disease, while it is possible that some, with a value above 40, may become affected. (3) The deficiency of protein may be: (a) *Primary*, in which the supply is insufficient for the individual requirement or, when, owing to the indigestible character of the food, a somewhat restricted supply cannot be utilized to the normal extent. (b) *Secondary*, in which owing to digestive disturbances, or other causes, the supply of protein cannot be assimilated. (4) In accordance with conclusion 3, three types of pellagra may be distinguished etiologically: (a) The common type seen in poverty or deficient food from other causes. (b) Relapse cases in which, owing to permanent defects—the result of a previous attack of pellagra—the protein supply is inefficiently utilized. (c) Cases in which a disease of the digestive organs due to other causes than insufficient feeding, leads to defective utilization of the protein intake. (5) That indicanuria is an important indication of the loss of protein in the intestine, the amount present being sometimes sufficient to account for the loss to the body of a large proportion of the protein intake. (6) Indicanuria is closely related to the deficiency of gastric hydrochloric acid. This is due to two causes: (a) The resulting invasion of the intestine with bacteria. (b) The absence of the normal stimulus to the secretion of pancreatic juice, whereby less protein is completely digested and more is lost in the large intestine. (7) Labor raises the level of protein requirement, an effect which is increased greatly by a deficient energy supply. Labor is therefore a factor in the causation of pellagra in a community whose protein supply is on the border-line between sufficiency and insufficiency. (8) There



is evidence which seems to suggest that a deficiency of cholesterol may be related to some of the symptoms."

Voegtlin<sup>2</sup> has prepared a map showing the present-day geographical distribution of pellagra, and Roberts<sup>3</sup> states that, although exact statistics are not available, there have been in this country from 1902 to 1920, 50,000 deaths from this disease. In 1916 pellagra ranked fourth as a cause of death in Mississippi, third in Alabama, and second in South Carolina. The most severe years since 1900 have been those between 1911 and 1916. There has been since 1917 a marked decrease both in prevalence and in severity of the disease. In some communities the decrease in number of cases has amounted to seventy-five per cent. Roberts thinks that, while there is a tendency to decrease in the number of cases, there is a tendency to increase in chronicity. According to this well-known authority on this disease, pellagra as seen in the South today is in most instances exceedingly mild compared with the same disease as seen twenty years ago. Acute pellagra is becoming rare, while mild cases are more in evidence. Bigland,<sup>4</sup> basing his observations upon Turkish prisoners seen during the War, concludes that pellagra is most frequently seen among the underfed, but is occasionally seen among the well-fed. In the latter instance, although the food eaten is in proper quality and quantity, there is something wrong with its digestion, absorption or assimilation. Enright<sup>5</sup> bases his statements upon studies of the disease as seen among German prisoners who were captured in the East and held in Egypt. Enright thinks that there is something more than a dietary deficiency in the causation of this disease. He found that the German prisoners apparently had an abundant ration and were not subjected to any undue hardship. He asks himself why, under these conditions, the German prisoners suffered from pellagra while the English soldiers, with no better food and doing much harder work, escaped. Enright's explanation when he attempts to answer this question is not very satisfactory. He is sure there was no food deficiency and he is inclined to the opinion that there must have been something wrong with the internal secretions, but what secretion was involved and what was wrong with it he is unable to tell. Year by year, and with the accumulation of observations, it seems that the weight of evidence showing that pellagra is due to an unbalanced diet, and especially to one poor in proteins of high biologic value, grows, and we are ready to agree with Voegtlin, who says: "(1) The hypothesis that there is a causal relation between pellagra and a restricted vegetable diet has been substantiated by direct proof to this effect and has led to results of considerable practical and scientific value. (2) The metabolism in pellagra shows certain definite changes from the normal, which point to decreased gastric secretion and increased intestinal putrefaction. (3) In the treatment and prevention of pellagra, diet is the essential factor. The disease can be prevented by an appropriate change in diet without changing the other sanitary conditions. (4) A diet of the composition used by pellagrins prior to their attack by the disease leads to malnutrition and certain pathologic changes in animals, resembling those found in pellagra. A typical pellagrous dermatitis

has not been observed in animals. Pellagra symptoms have been produced in man by the continued consumption of a restricted vegetable diet."

Roberts emphasizes the importance of rest and diet in the treatment of this disease, and suggests the alterative prescription of food, fat, and flat.

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—V. C. V.

# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, AUGUST, 1922

No. 11

## ORIGINAL ARTICLES

### THE DETERMINATION OF LACTIC ACID IN BLOOD\*

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WITH THE ASSISTANCE OF MISS M. E. ARMOUR

DURING the past few years we have had occasion frequently to determine the amount of lactic acid in blood, and occasionally in urine. We have used the method of Von Fürth and Charnass† but have found that there are various parts of the process in which considerable errors are liable to be made unless great care is exercised. In the present paper we are recording some of our experiences with the method as used with the modifications which we have introduced to avoid error. The method is inapplicable for urine without very considerable modifications and even with these the results, in our hands, were not very satisfactory. The present paper relates to the method for blood.

Since the method is apparently not well known among British and American workers we will first of all describe it in detail as used in this laboratory. A flask containing 100 c.c. of 2 per cent HCl is weighed, and from 25 to 50 c.c. blood is delivered into it, after which it is immediately reweighed and sufficient of a 5 per cent solution of  $\text{HgCl}_2$  added to precipitate all the proteins. We have found that 80 c.c. of this solution is necessary for 50 grams of blood. When less blood is taken, proportionately less  $\text{HgCl}_2$  solution should be used, so as not to increase the bulk of the  $\text{HgS}$  precipitate formed in the next stage. The mixture is made up to 250 c.c. with water, and is well shaken. After precipitation, or in about 30 mins. it is filtered through thin folded filter paper until as much filtrate comes through as possible. The excess of mercury is then precipitated by hydrogen sulphide, the precipitate removed by filtration and

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†Von Fürth and Charnass: *Biochem. Ztschr.*, 1910, xxvi 199, also *Analyse des Harns* (Wiesbaden), 1910, i. 233.

the filtrate accurately measured. An aliquot part, 175 c.c. (7/10ths) of the original solution can usually be secured, is taken for further analysis. This measured portion is then almost neutralized towards litmus with sodium hydroxide 4 per cent and placed in a large flat porcelain photographic developing tray with a lip at one corner. The bulk is reduced by evaporation in a warmed air stream. We have found that a large sized majestie heater working in line with a large sized office fan, quite rapidly reduces the solution. It is very important that the temperature during evaporation should never rise above 40° C. since above this temperature, even in faintly acid solutions, there is a decided loss of lactic acid. Under the above conditions, because of the rapid evaporation, the temperature rarely rises above 20° C.

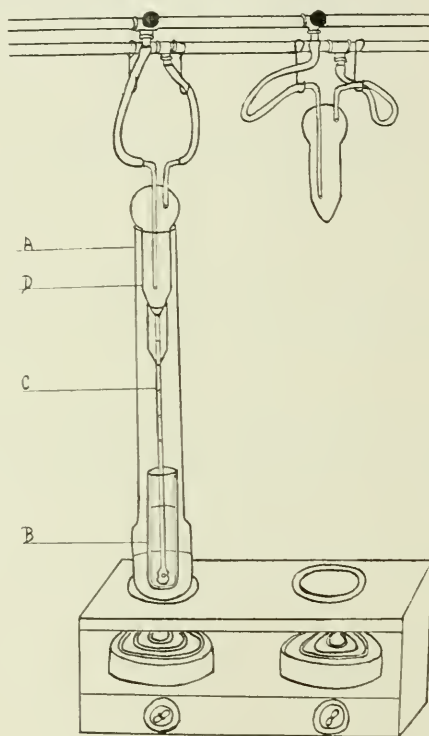


Fig. 1.

Evaporation is allowed to proceed until the volume is reduced to 50 c.c. (2 to 3 hours) and this is then transferred to the extraction tube of the Dunbar apparatus (*vide infra*). The photographic dish is washed into the tube by means of about 25 c.c. of saturated solution of Ammonium sulphate in three portions, and the fluid in the tube is saturated with ammonium sulphate (about 50 grams), 3 c.c. phosphoric acid added, and extraction is allowed to proceed for 48 hrs.

The Dunbar apparatus (see Fig. 1) consists of an outer glass cylinder bulged below (A), an extraction tube (B) having a loop of thin aluminum wire attached at its upper end, a thistle tube with a long stem (C) closed below except for several pin holes, and a conical condenser (D) which rests on the



upper edge of the outer tube. A sufficient amount of glass wool is tied round the lower end of tube C (by means of thread) so that it may serve to impede the upward passage of the ether as it comes from the openings at the end of the tube. Ten c.c. approximately N/10 NaOH solution and 200 c.c. alcohol-free ether are placed in A, and B is then carefully lowered into A by means of the loop of wire. The total fluid in B should not occupy more than three-fourths of the tube. The condenser is put in position and the assembled apparatus placed on an electric heater enclosed in an asbestos box with an opening to fit the lower bulged end of A. The degree of heat is regulated so that the condensed ether drops from the tip of the condenser in an almost continuous stream into the thistle funnel.

The ether used must be free of all traces of alcohol. For the purpose of purifying it commercial ether is shaken several times in a separator with 60 per cent KOH, then washed with water till the washings are neutral, and placed over  $\text{CaCl}_2$  for a day or so, and finally distilled. This purification is unnecessary when the ether distilled off from previous extractions is used.

When extraction is complete, B is partly removed from A by the wire loop, and C slowly lifted out, allowing every drop of ether to drip from it. The remaining ether in B is caused to overflow into A by cautiously adding water. The contents of A, which should be alkaline, are now carefully transferred to an Erlenmeyer flask connected with a condenser. The flask is placed on a water-bath heated by an electric lamp, the temperature of the water-bath during distillation never being allowed to rise above  $50^\circ \text{C}$ . Distillation is continued until no more ether distills over. The flask containing the faintly alkaline water solution of lactate is placed for a few minutes on a boiling water-bath so as to remove all traces of ether, after which the contents are carefully transferred to a one litre fractional distillation flask, being washed into this by means of 300 c.c. of solution (0.5 per cent) of  $\text{H}_2\text{SO}_4$ . The mouth of the flask is then closed with a rubber stopper through which passes the tube of a stoppered funnel, the lower end of the tube being drawn out to a fine point which extends close to the bottom of the flask. This funnel and tube are filled with potassium permanganate solution by suction, care being taken that no air bubbles remain in the tube. The side tube of the flask is so arranged that it can readily be connected with a worm condenser to the delivery end of which is attached a tube which pierces a large rubber stopper fitting a cylinder of about 400 c.c. capacity. This cylinder stands in a vessel of cold water, and to it is added 10 c.c. of approximately N/5  $\text{KHSO}_3$  solution (1.2 grams per 100 c.c.) and 10 c.c. of distilled water. The condenser tube is connected with a glass tube which dips into the bisulphite solution. A trap containing a few c.c. of water is inserted through a second hole in the stopper.

The contents of the distillation flask are now heated, and when boiling commences and steam emerges from the end of the side tube, connection is made between the side tube and the upper end of the condenser. The tap controlling the flow of the permanganate solution is now turned so that the permanganate solution flows at such a rate that the pink color of the solution emerging from the fine point of the tube disappears before reaching the surface of the solution

in the flask. The flame under the flask is now turned down slightly, so that it may be increased in case of back suction. We have found it most important to start the flow of permanganate very slowly, as otherwise the solution changes color too rapidly, and lactic acid is lost. The permanganate is added until a permanent pink (brownish) remains throughout the solution. The tap is then closed and boiling continued for ten minutes more. If no change takes place in the color, oxidation is complete. If otherwise, more permanganate must be added. When oxidation is complete, the side arm is disconnected from the condenser (before turning out the flame).

The condenser and trap are washed into the cylinder with distilled water by means of a water bottle, and the contents titrated against standard iodine (about N/10), starch solution being used as an indicator. It is also necessary with each fresh batch of ether to evaporate the amount (200 c.c.) used for extraction and to oxidize the residue in the flask exactly as in the estimation itself. This blank usually yields a small amount of distillate capable of combining with the bisulphite, but it should never exceed 1 c.c. N/25 iodine solution.

#### THE TITRATIONS

The following solutions are necessary:

1. *Deci-normal sodium thiosulphate* 24.8 gms ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) per litre. This is titrated against N/10 potassium dichromate which can readily be prepared by direct weighing from the fused salt. For this purpose about 6 gms. C.P. potassium dichromate is gently heated in a porcelain dish so that it becomes fused. The fused salt is then quickly powdered in a thoroughly dry mortar and placed in a desiccator. 4.913 grams are accurately weighed out and dissolved in 1 litre. To titrate the thiosulphate, 25 c.c. of N/10 dichromate are placed in a stoppered flask, about 2 gms. Pot. Iodide added and then about 5 c.c. C.P. HCl. The bottle is stoppered and allowed to stand for about 10 minutes in a warm place after which it is cooled. The stopper is removed and washed with water into the bottle. The thiosulphate solution is then run from a burette into the bottle until the brown color due to free I. is almost discharged. A few drops of starch solution are added and the titration continued until the blue color is completely discharged. The exact amount of thiosulphate is noted and the normality factor calculated.

2. *Approximately N/25th I-solution*—5 gm. I are placed in a litre flask. Fifteen gms. potassium iodide are dissolved in 200 c.c. water and this solution added to the flask which is then shaken until the I. dissolves. The volume is then made up to 1 litre.

3. *Potassium Bisulphite solution.* Dissolve 1.2 gms. Pot. Bisulphite in 100 c.c. water. This solution should be freshly prepared every two or three days.

4. *Approximately N/10 Potassium Permanganate.* (3.16 gms. per litre.) This is a stock solution from which the solution to be used for oxidizing is prepared. When large amounts of lactic acid are expected a N/100 solution is used. For smaller amounts, as in blood a N/300 is used (i.e. 100 c.c. N/10 stock solution diluted to 3 litres).

5. *Sulphuric acid solution.* 5.0 c.c.  $\text{H}_2\text{SO}_4$  (con.) in 1 litre water.

6. *One per cent starch solution (soluble starch).*

The routine of an analysis is as follows:

1. Titrate Thiosulphate solution with dichromate. (This need not be done every day.)
2. Titrate 10 c.c. I-solution with Thiosulphate.
3. Titrate 10 c.c. Bisulphite solution against I. (In both these titrations the brown color of the iodine serves as an indicator until near the end point when starch solution is used.)
4. Place 10 c.c. Bisulphite solution in receiving flask of oxidizing apparatus and proceed with oxidation as described on p. 636.
5. Remove flask and titrate distillate with I-solution.
6. Repeat 4 and 5 with ether residue as described on p. 638.

7. The difference between titrations 3 and 5 gives the number of cubic centimetres of I-solution which corresponds to the bisulphite that has combined with the aldehyde liberated by oxidation from lactic acid. The normality of this amount of I. is readily determined from titration 2 (by multiplying it by the value found in 2).

*The calculations are as follows:*

Each c.cm. N/10 Iodine solution corresponds to 0.5 c.c. N/10 Lactic acid. Therefore if we multiply the value found in the paragraph 7 above by 4.5 it will give the milligrams of lactic acid that have been oxidized. Since the titre of the I. against Thiosulphate solution remains tolerably constant from day to day, it is convenient to use a factor which consists of 4.5 multiplied by the c.c. N/10 Thiosulphate required to combine with 10 c.c. I-solution. This factor multiplied by the difference between the titrations 3 and 5 above gives mg. lactic acid.

To calculate the percentage it is then necessary to allow for the amount of blood and aliquot of filtrate used. The following is an example: Blood used 36.6 c.c.

Aliquot of filtrate used 178 c.c.

(1) 25 c.c. N/10 Dichromate solution = 25.3 c.c. Thiosulphate.

∴ Normality factor for Thio. = 0.988 (0.99).

(2) 10 c.c. I-solution = 3.7 c.c. N/10 Thiosulphate.

(3) 10 c.c. Bisulphite = 26.7 c.c. I-solution.

(5) After distillation = 14.5 c.c. I-solution.

Difference  $\frac{12.2}{}$

(6) Blank for ether  $\frac{.5}{}$   
11.7

The equation is: 
$$\frac{11.7 \times 3.7 \times 0.0045 \times 236.6 \times 10 \times 100}{178 \times 36.6} = 0.0707 \text{ per cent.}$$

*The following tests have been made of the degree of accuracy of the method.*

There are at least four stages in the process as outlined above at which losses of lactic acid may occur, (a) in the removal of the proteins from the blood, (b) in the evaporation to small bulk of the protein-free filtrate, (c) in the ether extraction (d) in the oxidation of the lactic acid contained in the final extract.

The first to consider is the oxidation process. It has been pointed out by von Fürth and Charnass that the concentration of the permanganate solution must not exceed 0.01 N. Using this strength these workers succeeded in recovering with tolerable constancy 92 per cent of lactic acid from lithium lactate. By no modification of the apparatus or strength of reagents could they obtain greater yields, and if the permanganate solution were made decidedly stronger, these became less satisfactory. On various occasions during the past few years we have had occasion to test this stage of the process and have found that although yields of approximately 92 per cent are certain when considerable quantities of lactic acid are oxidized by means of 0.01 N permanganate; this is not the case when small quantities are used.\* This is shown in Table I.

TABLE I

LACTIC ACID USED (AS LITHIUM LACTATE)	LACTIC ACID RECOVERED (AS ALDEHYDE)	PERCENTAGE OF LACTIC ACID RECOVERED
0.191 gms.	0.174	91.1
0.099	0.083	82.9
0.0706	0.0593	84.0
0.0202	0.0169	83.6

On the other hand when N/200 to N/300 permanganate is used, and care is taken that the fluid in the oxidation flask does not become tinted pink throughout, equally satisfactory results are obtained with very small amounts of lactic acid (Table II).

TABLE II

LACTIC ACID USED (AS LITHIUM LACTATE) (GM.)	LACTIC ACID RECOVERED (AS ALDEHYDE) (GM.)	PERCENTAGE OF LACTIC ACID RECOVERED (PER CENT)
0.01935	0.01755	90.7
0.0125	0.0114	91.2
0.0127	0.0112	88.1
0.0244	0.0224	91.6
0.0155	0.0142	90.1
0.0155	0.0142	90.1
0.0155 (after solution stood 16 hours)	0.0138	89.3

Concerning the *ether extraction* by the Dunbar apparatus, we have found that if this is continued for forty-eight hours with the ether dropping in an almost continuous stream from the condenser, the lactic acid is satisfactorily extracted. It is important, however, to see that a sufficient layer of ether forms on the top of the inner tube, so that none of the underlying watery solution is carried over into the outer tube. Sometimes large bubbles of ether vapor form at the tip of the delivery tube of the funnel, and by passing through the solu-

\*The lithium lactate used in these observations was prepared by saturating a solution of lactic acid with zinc carbonate (75 gm.  $\text{ZnCO}_3$  per 100 gm. lactic acid BP or USP) at boiling temperature, filtering and collecting the crystals of zinc lactate on a Buchner funnel. After recrystallization, the crystals were dissolved in water, the solution boiled and lithium carbonate added in small quantities at a time until a filtered portion of the mixture gave no cloudiness with  $\text{H}_2\text{S}$ . The mixture was then filtered and the filtrate concentrated until lithium lactate began to separate out, when it was cooled and the crystals separated by suction and dried in a vacuum desiccator. The lithium in the crystals was determined as  $\text{Li}_2\text{SO}_4$  by cautious incineration in a weighted crucible, a drop of  $\text{H}_2\text{SO}_4$  (con.) being added after most of the organic matter had been burned off.



tion in the inner tube so agitate it that some of this is inevitably carried over the edge of the tube. When this happens, the perforations in the delivery tube should be made larger. In the following observations quantities of lithium lactate of known strength were extracted, the ether evaporated off and the residues then oxidized as above described.

TABLE III

LACTIC ACID USED (AS LI. LACT.) (GM)	LACTIC ACID RECOVERED (AS ALDEHYDE) (GM)	PER CENT RECOVERED	TIME EXTRACTION HOURS
0.0225	0.0230	excess	46
0.0261	0.0236	90.4	48
0.0266	0.0231	86.7	96
0.0406	0.0370	91.2	72
0.0525	0.0475	85.2	72
0.0244	0.0224	91.6	46
0.0237	0.0194	81.8	72
0.0990	0.0890	90.	55

Although more observations are necessary to make it certain that forty-eight hours is sufficient time for tolerably complete ether extraction, we have chosen this period since we have not found that any greater yield of lactic acid is obtained by longer extraction.

The most serious losses are incurred in *the process of evaporation* of the blood filtrates. Although these are carefully kept at faintly acid reaction, and the temperature never permitted to rise above 40° C., some loss seems unavoidable and unfortunately one cannot be certain of its exact magnitude. Nor can this error be obviated by making the filtrates faintly alkaline, because of the sugar which they contain.

The following observations will illustrate the extent of the losses due to this cause, 50 c.c. of a solution of lithium lactate in distilled water was allowed to stand at room temperature, and at the periods indicated 10 c.c. portions were oxidized.

11:20 A.M.	10 c.c. contained	.0150 gm. lactic acid
2:20 P.M.	“ “	.0142 “ “ “
4:20 P.M.	“ “	.0142 “ “ “
10:00 A.M.	“ “	.0138 “ “ “

This observation was repeated, except that the solution of lithium lactate was dissolved in N/10 HCl with the following results:

10:20 A.M.	10 c.c. contained	.0243 gm. lactic acid
3:15 P.M.	“ “	.0239 “ “ “
10:00 A.M.	“ “	.0229 “ “ “

A distinct decrease is observed in the amount of lactic acid in both cases. We have not determined to what extent this diminution in the amount of recoverable lactic acid would go after prolonged standing in acid solutions, but we have frequently observed that solutions in distilled water show marked deterioration in yield after standing some weeks. Thus, 3 c.c. of a solution of li. lact. made in November, 1921, contained 0.01775 gm. lactic acid, and in Jan., 1922, only 0.0120 gm.

In view of this deterioration, the cause for which we have not investigated, we have always used, for purposes of testing the accuracy of the method, freshly prepared solutions of lactate made up from li. lactate kept in a desiccator.

These losses become still greater when the solutions have to be evaporated, even although the temperature may not be allowed to rise above 30° C.

Quantities of lithium lactate were dissolved in about 200 c.c. distilled water (corresponding to the volume of blood filtrate usually employed), made faintly acid, evaporated in the air current to a volume of 50 c.c., then transferred to the oxidation flask and oxidized with permanganate.

TABLE IV

CONDITION OF LACTATE SOLUTION	AMOUNT LACTIC ACID PRESENT TO START (AS LI. LACTATE)	AMOUNT OF LACTATE ACID RECOVERED (AS ALDEHYDE)	PERCENTAGE RECOVERED
Distilled water	0.0529	0.0379	72
Distilled water	0.0286	0.0235	82.2
Faintly acid	0.0276	0.0223	81
Faintly alkaline	0.0328	0.0265	80.7
Distilled water	0.0300	0.0236	79
Decidedly acid	0.0200	0.0140	70
Decidedly acid	0.0399	0.0282	70.6
Distilled water	0.0197	0.0181	83.2
Faint acid with phosphate buffer	0.0278	0.0247	88.8
Solution of phosphate buffer	0.0155	0.0121	84.7

A part of these considerable losses in the evaporation process may be due to mechanical causes, although we have used every precaution to avoid this. The employment of a buffer mixture of phosphates seems to minimize them and we regret that this precaution had not been adopted earlier in our work.

Realizing that with every precaution, and with precise standardization of the quantities of reagents used, the method is nevertheless fraught with many sources of error, we have from time to time tested its degree of accuracy by adding li. lactate to blood. This test involves an estimation of the lactic acid originally present in one sample of blood and that in another sample to which li. lactate has been added. Some of the more recent results are shown in Table V.

TABLE V

LACTIC ACID PRESENT IN ORIGINAL BLOOD (PER CENT)	LACTIC ACID ADDED (PER CENT)	LACTIC ACID RECOVERED (PER CENT)	DIFFERENCE BETWEEN 1 AND 3	PER CENT OF ADDED LACTIC ACID RECOVERED
1	2	3	4	
74 0.087	0.031	0.116	0.029	93
75 0.087	0.116	0.186	0.099	85.3
31 0.062	0.129	0.175	0.113	87.0

## THE THERAPEUTIC USE OF GERMANIUM DIOXIDE IN ANEMIA\*

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THE erythropoietic action of germanium was first demonstrated by Hammett, Nowrey and Müller,<sup>1</sup> who showed that after subcutaneous injections of germanium dioxide solution into mature albino rats, there was a prompt and marked rise of 1 to nearly 5 million erythrocytes per cu. mm. of the rats' blood. No leucemia was produced as an accompaniment of the erythrocytosis. A similar effect in the guinea pig, rabbit, dog and man was demonstrated by Müller and Iszard,<sup>2</sup> who noted a certain periodicity in the erythrocyte curves. The close chemical relationship between arsenic and germanium in the periodic system originally suggested to these investigators the possibility that germanium might have some effect on erythrocyte formation, a possibility which appears to be substantiated by their findings.

It was shown that the germanium does not accumulate, but is eliminated chiefly through the kidneys and in small part by the alimentary tract.<sup>2</sup> Up to 180 mg. germanium dioxide per kilo body weight, may be given to the albino rat with no apparent harmful results.<sup>3</sup> However, relatively large doses were found to be distinctly toxic to guinea pigs, the lethal dose being about 586 mg. germanium dioxide per kilo body weight. This toxic action may possibly be due to an overstimulation of the blood forming organs.<sup>2</sup> The source of the erythrocytosis appears to be an increased production of red cell precursors in the bone marrow stimulated to greater activity by the germanium.<sup>4</sup>

As a result of these observations it has been suggested that germanium may prove to be a therapeutic agent of considerable value in the treatment of anemia. The subject is one that has aroused considerable interest, although no clinical studies have as yet been reported. The present paper shows the results we have obtained on a series of unselected cases of various types of anemia.

### ADMINISTRATION

We have treated sixteen patients suffering from anemia, administering the germanium by mouth in 0.2 per cent water solution. In order to dissolve the germanium dioxide (N. J. Zine Co. product) it was found necessary to boil the solution for several minutes; the oxide does not precipitate out on cooling. Doses between 100 and 200 mg. of the oxide were given daily or every 2 or 3

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A preliminary report of these observations was made to the Society for Experimental Biology and Medicine, May 17, 1922, Proc., xix, 398.

Received for publication, July 20, 1922.

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days until about 1 gram had been given. The actual amounts administered may be seen in the table.

#### METHODS EMPLOYED

Erythrocyte counts were generally made daily during the period of germanium treatment, and for some time afterwards, with counts every few days or weekly later on. Blood counting pipettes and hemacytometer with the double Neubauer ruling, certified by the U. S. Bureau of Standards, were used. Hemoglobin determinations were made by treatment of the blood with 0.1 N hydrochloric acid, allowing it to stand for several hours (Robscheit<sup>5</sup>) to form acid hematin, and comparing against a standard in a Bock-Benedict colorimeter. The standard of acid hematin was prepared from blood whose oxygen capacity was determined by the method of Van Slyke.<sup>6</sup>

The percentage of total solids in the blood was determined<sup>7</sup> to see whether an observed increase in erythrocyte count was real, from an increased production of cells, or only apparent, due to concentration of the blood by decrease in plasma volume, caused by loss of fluid from the blood. If an increase of 25 per cent noted in the red cell count is only apparent, due to decrease in plasma volume, there should be a corresponding increase in the total solid content of the blood. Since finger blood was used and a number of determinations were made, the amount of blood available for total solids, generally less than 100 mg., was not so large as is desirable. Determinations of the leucocyte and differential white cell counts were made several times on most of the patients, while the coagulation time of the blood was determined in several instances. The Wassermann test was done in certain cases before and after the treatment with germanium dioxide. Routine urine analyses, and chemical blood determinations of the urea, uric acid, sugar and chlorides were made before and after the germanium treatment, on certain patients, to determine whether the medication had any harmful effect on the functional efficiency of the kidneys.

#### DISCUSSION

The data on red cell counts, hemoglobin and total solids in blood are given in the table. The amounts of these constituents in the blood are shown, as well as the percentage of change from the average of several counts made before the germanium was given. The increases in total solids generally run parallel to the hemoglobin changes, but are not sufficiently great to explain the large increases in erythrocytes on the grounds of decrease in plasma volume. In view of the histological findings of Hammett,<sup>4</sup> who showed that many more nucleated erythrocytes were present in the bone marrow of the germanium treated rats, than in the marrow sections of the controls, and that the blood of the test rats contained more young red cells than did the blood of the controls, we feel that our data on total solids strengthen their conclusion that germanium dioxide produces a real erythrocytosis.

The results of germanium treatment on these patients are presented for the purpose of stimulating further study of the problem, and with no attempt to draw general conclusions as to the therapeutic value of the compound, since the number of cases we have studied is so small.



TABLE I

## EFFECT OF GERMANIUM DIOXIDE ON THE BLOOD IN ANEMIA

CASE	DATE 1922	GERMANIUM DIOXIDE MG.	BLOOD					
			ERYTHROCYTES		HEMOGLOBIN		TOTAL SOLIDS	
			Million per cu. mm.	Percentage increase	Gms. per 100 c.c.†	Percentage increase	Gms. per 100 c.c.	Percentage increase
1. S. L.	4-12*	100	3.50		11.2		14.7	
	4-14	100	3.86	13	12.1	7	18.0	20
	4-17	150	3.81	12	14.4	28	20.0	30
	4-19	200	4.35	28	13.6	20		
	4-21	200	4.46	31	14.4	28		
	4-24	150	4.78	40	15.4	36	18.9	26
	4-26	200	4.62	36	15.2	35	20.2	35
	4-28		5.00	47	15.4	36	19.3	29
	5-1		5.18	52	14.8	31	19.8	32
	5-5*		5.74	68	17.3	53	21.4	43
	5-8		5.26	54	17.1	51	21.6	44
	5-15		6.05	77	15.9	40	19.7	31
	6-2*		5.90	73	19.4	72		
	6-11		6.09	79	19.4	72		
2. C. B.	4-14*	100	2.02		7.0		13.2	
	4-15	100	2.43	15	7.9	12		
	4-16	100						
	4-17	100	2.18	3	7.9	12		
	4-18	150	2.33	10	7.4	4		
	4-19	150	2.32	10	7.9	12		
	4-20	200	2.70	28	8.0	13	15.7	19
	4-21	200	2.48	17	8.1	15	15.0	14
	4-22	200	2.46	17	8.1	15		
	4-25*		3.09	46	8.2	16		
	5-1*		3.62	71	9.5	35		
	5-16*		3.33	58	8.7	24		
	5-19	200	3.78	79	8.8	25		
	5-20	200			8.3			
	5-21	200						
	5-22	200	3.69	75		17	15.2	13
	5-24	200	3.46	64				
	5-29		3.89	85	9.3	32	16.0	21
	6-1		3.93	86	8.5	21	16.8	27
	6-9		3.64	73	8.5	21	14.3	8
3. I. S.	5-16*	150	4.57		10.2		17.2	
	5-17	150						
	5-18	150						
	5-23	150	4.73	4	10.0	-2	15.4	-10
	5-24	150						
	5-25	150						
	6-7	150	4.94	8	11.0	8		
	6-14		4.59	0.5	11.1	9	17.7	3
4. F. D.	4-1*	100	2.98		9.0		13.0	
	4-2	100	3.49	18	8.8	-3	11.5	-12
	4-3	150	3.42	16	9.2	3	13.8	6
	4-4	150	3.17	7	8.9	-2	14.4	11
	4-5	150	3.20	8	8.8	-3	12.5	-4
	4-6	150	3.23	10	8.9	-1		
	4-7	150	3.01	2	8.9	-1		
	4-14*		3.55	20	9.7	8		
	4-28		2.67	-10	6.2	-31		

†The average normal hemoglobin content for women is 15.5 grams, for men, 17 grams, and for children, below 14 grams per 100 c.c. blood.

\*To save space, one or more determinations of blood constituents made before this date are omitted from the table.

TABLE I—CONT'D

## EFFECT OF GERMANIUM DIOXIDE ON THE BLOOD IN ANEMIA

CASE	DATE 1922	GERMANIUM DIOXIDE MG.	BLOOD					
			ERYTHROCYTES		HEMOGLOBIN		TOTAL SOLIDS	
			Million per cu. mm.	Percentage increase	Gms. per 100 c.c.†	Percentage increase	Gms. per 100 c.c.	Percentage increase
	5-2	200						
	5-3	200						
	5-4	200						
	5-5	200						
	5-6	200	3.63	24	11.7	30		
	5-8	200						
	5-13		4.13	42	14.1	57		
5. S. C.	4-29*	100	4.10		13.6			
	5-1	100	4.17	—4	14.1	—3		
	5-2	150	3.93	—9	15.0	4		
	5-3	150						
	5-4	150	4.58	6	15.9	10		
	5-5	150						
	5-6	100	4.85	12	15.0	4		
	5-8	200	5.34	23	15.2	5		
6. J. S.	6-7*		3.85		10.6			
	6-9	150						
	6-10	150			10.4	—2	18.0	
	6-11	Less than 150						
	6-12	Less than 150						
	6-14		4.59	21	11.5	9	15.9	
7. J. M.			4.51	19	10.9	3	16.2	
	4-17*	100	3.94		12.8		18.7	
	4-19	100	3.94	—3	12.9	1	16.5	—12
	4-21	150	4.13	3	13.1	2	17.9	—5
	4-24	150	4.26	5	14.1	10	17.7	—6
	4-26	150	4.45	10	13.2	3	18.7	0
	4-28	150	4.70	16	14.1	10	18.0	—4
	5-1	150	4.52	12	13.6	6	18.4	—2
	5-3	200	4.38	8	13.1	2	19.3	3
	5-10*		5.10	26	13.7	7	20.9	12
	5-26		4.06	0.4	13.6	6	18.5	—1
	6-7		4.30	6	12.7	—1	18.1	—3
8. I. N.	3-21*		3.84		6.8		13.2	
	3-22	100						
	3-23	100	4.45	18	7.0	3	12.4	6
	3-24	150	4.86	30	7.2	6	13.2	0
	3-25	150	4.99	33	7.4	9	15.1	14
	3-26	150	4.80	28	7.3	7		
	3-27	150	5.18	38	7.4	9		
	3-28	150	4.70	25	7.1	4	14.3	8
	3-29		5.41	44	7.4	9	13.0	—2
	4-2*		4.83	29	7.4	9	13.4	2
	4-4*		5.76	53	7.8	15	15.8	20
	4-15*		5.18	38	7.6	12	13.6	3
	4-22		4.05	8	7.3	7	14.1	7
	4-29	150	4.32	15	7.4	8	12.9	—2
	5-1	150	4.56	21	7.6	12	14.4	9
	5-3	150	4.15	11	7.0	3	14.5	10
	5-8	150	4.68	25	6.9	2	10.6	—20

†The average normal hemoglobin content for women is 15.5 grams, for men, 17 grams, and for children, below 14 grams per 100 c.c. blood.

\*To save space, one or more determinations of blood constituents made before this date are omitted from the table.

TABLE I—CONT'D

EFFECT OF GERMANIUM DIOXIDE ON THE BLOOD IN ANEMIA

CASE	DATE 1922	GERMANIUM DIOXIDE MG.	BLOOD					
			ERYTHROCYTES		HEMOGLOBIN		TOTAL SOLIDS	
			Million per cu. mm.	Percentage increase	Gms. per 100 c.c.†	Percentage increase	Gms. per 100 c.c.	Percentage increase
	5-11	150	4.54	21	6.9	2	13.6	3
	5-15	150	4.43	18	7.2	6		
	5-20*		4.25	13	7.9	16	14.4	9
	5-26*		4.23	13	7.0	4	13.3	1
	6-8*		4.01	7	7.5	10	13.0	—2
	6-13		3.78	0.5	7.5	11	15.0	14
	6-17		3.86	3	7.7	13		
9. W.	5-2	200	4.54		10.1		18.5	
	5-3	200						
	5-4	200						
	5-5	200	4.44	—2	9.5	—6	16.0	—13
	5-6	200						
	5-7	200						
	5-15*		4.22	—7	9.7	—4	19.1	3
	6-9*		4.36	—4	10.2	1	17.5	—5
10. E. A.	6-22		4.44	—2	11.0	9	17.7	—4
	4-13*	100	3.41		8.5		15.5	
	4-15	100	3.46	1	8.0	—5	13.2	—15
	4-17	100	3.32	—3	8.7	3	14.6	—6
	4-19	100	3.59	5	8.7	3	13.3	—14
	5-9		2.63	—23	6.3	—25	13.8	—11
	5-10	100	2.74	—20	6.3	—25		
		Less than						
	5-11	100						
	5-12	100	2.51	—26	5.9	—31	10.4	—33
	5-14	100	2.72	—20	6.3	—25		
	5-15	100						
	5-16		2.08	—39	5.8	—31	11.2	—28
	5-19		1.93	—43	5.2	—39	12.3	—21
	5-21		2.57	—25	5.8	—31		
11. L. M.	6-6*		3.34		9.3			
	6-8	150						
	6-9	150	3.30	0	9.3	1	17.4	
	6-10	150	3.53	7				
	6-11	150						
	6-12	150	3.26	—1	9.8	6	15.1	
	6-13	150						
	6-14	150	3.28	—1	9.3	1	15.3	
	6-21*		3.85	16	10.2	11	16.0	
	6-30		3.35	1	9.4	2	15.9	
12. W. U.	4-13*	100	3.84		14.8		19.4	
	4-14	100	3.71	—7	13.9	—8	18.8	1
	4-15	100	4.01	1	15.0	—1	17.7	—5
	4-16	100						
	4-17	100	3.80	—5	14.8	—2	17.6	—6
	4-18	150	4.22	6	15.0	—1	17.9	—4
	4-19	150	4.15	4	14.4	—5		
	4-20	200	3.99	0	14.6	—3	18.9	1
	4-21	200	4.19	5	13.7	—9	18.9	1
	4-22	200	3.90	—2	13.9	—8		
	5-8*		4.13	4	13.0	—14	19.9	6
	5-16*		3.68	—8	12.8	—16		

†The average normal hemoglobin content for women is 15.5 grams, for men, 17 grams, and for children, below 14 grams per 100 c.c. blood.

\*To save space, one or more determinations of blood constituents made before this date are omitted from the table.

TABLE I—CONT'D

## EFFECT OF GERMANIUM DIOXIDE ON THE BLOOD IN ANEMIA

CASE	DATE 1922	GERMANIUM DIOXIDE MG.	BLOOD					
			ERYTHROCYTES		HEMOGLOBIN		TOTAL SOLIDS	
			Million per cu. mm.	Percentage increase	Gms. per 100 c.c.†	Percentage increase	Gms. per 100 c.c.	Percentage increase
13. T. P.	6-14		2.11		7.8		13.3	
	6-15	150						
	6-16		2.22	5	7.6	—3	13.5	1
	6-17	150						
	6-18	150						
	6-19	150	2.03	—4	7.9	1	14.8	11
	6-20	150						
	6-21	150	2.16	2	7.5	—4		
	6-22	150						
	6-23	150	2.18	3	7.5	—4	14.5	9
	6-24	150						
	6-30		1.94	—9	7.3	—5	15.5	16
	7-3		1.94	—9	7.8	0	13.5	1
14. W. H.	7-10		2.70		10.4		16.6	
	7-11	150						
	7-12	150	2.37	—12	10.0	—4	14.5	—13
	7-13	150						
	7-14	150	2.52	—7	10.5	1	15.2	—8
	7-15	150						
	7-16	150						
	7-17		2.65	—2	11.2	8		
	7-19	200	2.48	—8	10.7	3	14.4	—13
	7-20	100**						
	7-21	100**						
	7-22	100**			10.4	0	16.5	—1
	7-24		2.40	—11	10.3	—1		
15. A. K.	7-5		2.02		4.6		11.3	
	7-7	25						
	7-8	35	2.05	1				
	7-9	50						
	7-10	50	2.33	15	5.1	10	11.8	5
	7-11	50	2.59	28	5.5	21		
	7-13	50	2.76	37	6.2	35	12.1	7
	7-14	50	2.62	30	6.0	30		
	7-15	50	2.81	39	6.1	32		
	7-16	50						
	7-17		2.75	36	6.1	32		
	7-19	50	3.00	48	6.2	35	11.6	3
	7-22		3.18	57	6.7	46	12.3	9
	7-24		3.21	59	6.6	44		
16. C. F.	7-7*	150	4.22		8.3		16.0	
	7-8	150						
	7-9	150						
	7-10		3.86	—9	8.5	2		
	7-11	100						
	7-12	150	4.52	7	8.7	5	15.4	—4
	7-14	150	4.86	15	9.2	11	14.7	—8
	7-15	150						
	7-17		4.64	10	8.3	0		

†The average normal hemoglobin content for women is 15.5 grams, for men, 17 grams, and for children, below 14 grams per 100 c.c. blood.

\*To save space, one or more determinations of blood constituents made before this date are omitted from the table.

\*\*In these instances a 0.2 per cent solution of germanium dioxide in 0.4 per cent sodium bicarbonate solution was used.



In the four cases of anemia following hemorrhage (cases 1-4 in Table) it is seen that the ingestion of the germanium was followed by prompt and large increases in both red cells and hemoglobin. The maximum increases in red cells were 79, 86, 8 and 42 per cent of the control counts, with increases up to 72, 35, 9 and 57 per cent above the control hemoglobins. In these cases there seemed to be little tendency for the counts to decrease to the original level after the effect of the treatment had worn off. While it is well recognized that after hemorrhage there is generally somewhat rapid regeneration of erythrocytes, germanium may prove to be of value in stimulating this regeneration.

CASE 1.—S. L., male; age thirty-five; March 14, 1922, a herniotomy was performed; March 24, hemorrhage from the lower bowel; diet, mixed.

CASE 2.—C. B., male; age twenty-four; weight 125 pounds; April 3, operation on fibrosarcoma of right antrum, hemorrhage at time of operation; diagnosis, fibroma of antrum, nose and nasopharynx; May 31, slight hemorrhage; treatment, May 31, 110 mg. of radium; diet of meat, eggs, milk, vegetables.

CASE 3.—I. S., male; age twenty-four; diagnosis empyema; April 28, resection of part of 6th rib; hemorrhage following operation; diet mixed; at time of germanium treatment patient was still discharging pus from pleural cavity.

CASE 4.—F. D., female; age forty-eight; diagnosis, carcinoma of body of uterus and cervix; October, 1921, hysterectomy was performed; treatment before germanium treatment, 10,000 mg. hours of radium; slight bleeding for some days beginning April 4; April 27, hemorrhage of about 1.5 ounces; treatment, beginning April 19, 1.5 ounces "ovoferrin" daily, and 1/4 gr. morphine daily; diet, soft, with eggs, milk, cereal, toast; death on May 13.

S. C. (Case 5), a woman with secondary anemia, visceroptosis and colitis, responded to the germanium treatment by a maximum increase of 23 per cent in red cells and 10 per cent in hemoglobin.

J. S. (Case 6), a woman who had dental caries, pyorrhea, and chronic arthritis, received four doses of germanium, but vomited part of the last two doses. She showed maximum increases in red cells of 21 per cent and of 9 per cent in hemoglobin. Unfortunately these two patients (5 and 6) were not studied after the treatment ended, and we are unable to tell whether the increase in red cells was permanent.

CASE 5.—S. C., female; age forty-seven; diagnosis visceroptosis, colitis and secondary anemia; diet mixed.

CASE 6.—J. S., female; diagnosis, dental caries, pyorrhea, chronic arthritis and secondary anemia; diet, soft; medication, 75 gr. sodium bicarbonate daily, May 15-20; 30 gr. tolysin daily May 20-June 6. Bland's pills, 6 daily, May 20-June 6.

J. M. (Case 7), a woman suffering from mucous colitis, tachycardia and secondary anemia, showed response to germanium by increases in red cells up to 26 per cent above the control; 3 weeks after the treatment the red cell count was 0.4 per cent above, and 2 weeks later, 6 per cent above the control. The maximum increase in hemoglobin was 10 per cent; 5 weeks after the treatment it had decreased to 1 per cent below the control.

I. N. (Case 8), a woman with secondary anemia, responded to germanium treatment with maximum increases of 53 per cent in red cells and 15 per cent in hemoglobin; twenty-four days after the last dose, the red cells had dropped to 8 per cent and the hemoglobin to 7 per cent above the original counts. A second treatment resulted in increases of 25 per cent in red cells and 16 per cent in hemoglobin; a month after this treatment the red cell count had decreased to 0.5 per cent. with a rise 4 days later to 3 per cent above the control. The hemoglobin at this time was 13 per cent above the control; a month earlier the patient had begun taking 1.5 ounces of "ovoferrin" daily, which may explain the fact that the hemoglobin remained at a higher level than the cells. The cause of the anemia in this case was unknown although there was a possibility of congenital syphilis.

In Cases 7 and 8 it appears that if the cause of the secondary anemia is not removed, the effect on the blood count of treatment with germanium may be relatively transitory. This type of secondary anemia, particularly, warrants further study with germanium.

CASE 7.—J. M., female, age thirty-five; diagnosis, mucous colitis, tachycardia and secondary anemia; diet, mixed.

CASE 8.—I. N., female; age nineteen; weight 114 pounds; diagnosis, secondary anemia, cause not determined, possibly congenital syphilis; blood Wassermann negative; diet, soft, with vegetables, meat and eggs; medication, beginning May 15, 1.5 ounces "ovoferrin" daily; June 13, teeth x-rayed; June 15, several teeth extracted, of which 3 were abscessed.

A woman, W., (Case 9), with carcinoma of the cervix, who was given germanium one week after the last radium treatment, showed decreases in both red cells and hemoglobin. The toxemia due to carcinoma, or the radium treatment may have counteracted the effect of the germanium in this case.

E. A. (Case 10), a girl with malignant endocarditis, streptococcus viridans type, showed small increases in red cells and hemoglobin after the first germanium treatment, but later, due to the progress of the disease, the counts had dropped decidedly, and a second treatment with germanium had little influence on the blood picture.

In the case of L. M. (Case 11), a girl suffering from malignant endocarditis, streptococcus viridans type, there were moderate increases and decreases in erythrocytes after germanium, with a maximum increase of 11 per cent in hemoglobin; a week after this the hemoglobin was 2 per cent above the control.

W. U. (Case 12) a man suffering from chronic cardiovalvular disease, reacted to germanium treatment with small increases and decreases in red cells, and decreases as great as 16 per cent below the control hemoglobin content.

T. P. (Case 13), a man suffering from pernicious anemia showed small increases and decreases in both erythrocytes and hemoglobin following germanium ingestion.

The treatment of a second case (W. H. 14) of pernicious anemia with germanium resulted in decreases in red cells, with small increases and decreases in hemoglobin.

Judging from the blood counts, Cases 9-14 showed practically no improve-

ment following germanium administration. Clinically there was little, if any, improvement. These are rather unfavorable cases, however, by which to judge any treatment, since the anemia was caused by pathological conditions which are difficult to remedy.

CASE 9.—W., female; age forty; diagnosis, carcinoma of uterus, cervix and anterior vaginal wall; treatment, 10,000 mg. hours of radium, last application on April 24; beginning May 8, 1.5 ounces "ovoferrin" daily; diet mixed.

CASE 10.—E. A., female; age thirteen; weight 70 pounds; diagnosis malignant endocarditis, streptococcus viridans type; diet, high caloric, and after June 9, soft.

CASE 11.—L. M., female; age twenty-one; diagnosis, malignant endocarditis, streptococcus viridans type; diet, high caloric; medication, 20 gr. tolysin daily, May 8-June 1; Bland's pills, 6 daily, June 1 to 6; Fowler's solution, 9 minims daily, June 1-6.

CASE 12.—W. U., male; age forty-five; weight 107 pounds; diagnosis, chronic cardiovascular disease, aortic regurgitation, mitral stenosis and regurgitation, myocardial insufficiency; diet, soft; medication, April 10-May 22, 45 minims tincture of digitalis daily.

CASE 13.—T. P., male; age fifty-three; weight 140 pounds; diagnosis, pernicious anemia; diet, high caloric; blood Wassermann negative, spinal fluid Wassermann positive; medication, neosalvarsan, 0.2 gm. May 22, 0.3 gm. May 29, 0.45 gm. June 5, 0.6 gm. June 12, 0.9 gm. June 20; mercuric chloride 1 gr. April 28, May 8, May 19; transfusion of 520 c.c. blood April 22, 560 c.c. May 3, 580 c.c. June 2.

CASE 14.—W. H., male; age fifty-six; diagnosis, pernicious anemia; five transfusions between September, 1921 and April, 1922; diet, mixed.

A. K. (Case 15), a boy aged 1½ years, who was convalescing from lobar pneumonia and parotitis, responded to treatment by prompt increases in red cells up to 59 per cent and in hemoglobin up to 46 per cent above the control.

C. F. (Case 16), a woman suffering from hemorrhoids, gave response to treatment by increases of 15 per cent in red cells and 11 per cent in hemoglobin. The effect of the germanium was somewhat influenced by slight bleeding.

CASE 15.—A. K., male; age one and one-half years; weight, 20 pounds; diagnosis, anemia, convalescence after lobar pneumonia and parotitis: diet, milk, vegetables, cereals, broth, bread.

CASE 16.—C. F., female; age thirty-two; diagnosis, hemorrhoids, internal, with slough, hemorrhoidectomy, July 18; diet, mixed.

By a study of the table it is seen that the periodic rise and fall of the red cell count after germanium, noted by Hammett and Müller, was also seen in this series of cases. The hemoglobin generally showed less of this periodic effect. The influence of germanium on hemoglobin production was usually somewhat less marked and more delayed than its influence on red cell formation; consequently, the color index usually dropped slightly early in the treatment and rose later.

Determinations of the coagulation time of the blood were made in 5 cases before and after the treatment, and revealed no noteworthy effect due to the germanium. There was no significant change in the white blood cell counts, nor in the distribution of different forms of white cells as shown by differential counts of the stained blood smears. From the results of the Wassermann test done in 4 cases before and after the treatment, it appears that the germanium did not affect the reaction of the blood to the test. A study of chemical blood

and urine analyses in 7 cases revealed no apparent effect of the germanium on the functional activity of the kidneys.

#### CONCLUSIONS

The influence of germanium dioxide on red blood cell formation has been studied in sixteen cases of anemia—four cases of anemia following hemorrhage, ten cases of secondary anemia and two cases of pernicious anemia. In some of these cases the germanium was found to have distinct erythropoietic action. Whether this property shall prove to be of great therapeutic value in the treatment of the anemias remains to be shown by further careful study of more cases than are here presented.

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### THE RELATION OF ACHYLIA GASTRICA TO CHRONIC FOCAL INFECTIONS AND PERNICIOUS ANEMIA\*

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THE high mortality of pernicious anemia warrants the presentation of any data which might enable a diagnosis of the latter disease to be made earlier or even suspect its oncoming. It is this motive which actuates the report of three cases of long continued focal infections passing through the stages of achylia gastrica, pernicious anemia, and death. The sequence of events whereby achylia gastrica preceded pernicious anemia in a chronological order in no way determines the former as an etiological factor of the latter, but it is the purpose of the writer in this connection to point out this possible relationship. The association of these two conditions has attracted the attention of clinicians for many years and efforts have been made to place the two conditions in the light of cause and effect. This attempt has not been successful and a careful analysis of all the data does not lead to the conclusion that such a relationship is at all probable.

Achylia gastrica is not a rare condition. It is a rather common finding and would be even more common were the gastric contents of patients routinely examined. The loss of gastric secretion may be complete or partial. Achylia may be associated with direct atrophy of the glands of the mucous

\*Received for publication, March 16, 1922.



membranes of the stomach or it may result from a depression of the function of the glands due to a large number of causes. It is associated with many toxic conditions, infections, nervous states, and vascular disturbances. It is stated by careful observers that total and persistent gastric achylia is consistent with perfect health and well being. However, such a condition must be unusual in comparison with the large number of cases associated with definite predisposing causes.

Fenwick reported the association of achylia gastrica and pernicious anemia as early as 1880 and since that day a voluminous literature has appeared. There has been a great diversity of opinion in regard to the relationship between this glandular degeneration or depression of function and the blood changes so that the question of cause and effect has been brought up. Many writers have considered the glandular degeneration as the primary factor and it is true that total atrophy of the gastric glands may cause serious disturbances of the general health. On the other hand, there is no question that complete loss of peptic power may be tolerated for many years without apparent impairment of the general health providing that the motor power of the stomach remains good, permitting the intestine to assume more or less vicariously the functions of the stomach.

Large numbers of cases of pernicious anemia have been subjected to gastric analysis in the earlier stages and it has been shown that the gastric mucous membrane is not always involved at this time, but as the disease advances and the anemia increases there is usually a corresponding decrease in the secretions of the stomach so achylia is nearly always present sooner or later. Comparatively little attention has been directed to the clinical fact that achylia gastrica may precede anemia in some cases for long periods of time. No importance is to be given to this association of events if it is assumed that these conditions are altogether separate and occur only as coincidental events. In fact, Stockton, editing Nothnagel's System of Medicine, states that when achylia precedes the pernicious anemia it is to be considered as coincidental. Exception may be taken to this on very definite grounds. There is reason to believe that the two conditions are manifestations of the same causative factors. It is not my purpose here to discuss the various agents assumed to be predisposing factors in the development of pernicious anemia. There is, however, an impression more or less general that pernicious anemia is a result of some infection in the body continuing over long periods of time, possibly for many years, so that the blood forming organs are damaged and rendered incapable of forming new cells. The fact that achylia gastrica may be due to similar states or infections in the body, whether in the tonsils, teeth, sinuses, gall bladder, prostate, or other places, has not been sufficiently emphasized.

Achylia gastrica is assumed in this connection to mean a condition of absent hydrochloric acid, as shown by congo red, with a total acidity that is very low or altogether absent. The gastric secretion is very susceptible to chronic infections of the body of whatsoever nature. It is absent in many conditions of the most diverse nature. My purpose in reporting three cases

of pernicious anemia which have been preceded by achylia gastrica for long intervals of time is not to insist that such a relationship is constant, but that when achylia is found in the presence of such infections it may be considered as a warning signal of a possibly oncoming pernicious anemia. The recognition of pernicious anemia in the stage of its incipency or in a stage before it is considered pernicious is of absolute importance in consideration of the hopeless prognosis in this disease. Despite the use of blood transfusions and other known therapeutic resources, the ultimate results in pernicious anemia are so uniform that any recognition or warning of the incipient stage of this condition is worth serious consideration. It is a relatively easy matter to determine the chemistry of the gastric contents, especially since the general use of the small duodenal tube. The use of this tube has made gastric analysis much more common and in view of the ease of introduction, it may be possible to detect these early cases in this way. Given a case of severe infection at the roots of the teeth, sinuses, tonsils, or other places, with slight grades of anemia, gastric analysis should be routinely made if there is any suggestion of digestive disturbances. The presence of an achylia in such a condition should at once throw the clinician on his guard because an infection which is severe enough to inhibit the secretion of the gastric mucosa may be severe enough in its toxic manifestations to result eventually in bone marrow changes with the evidences of a fatal anemia. There is no characteristic syndrome suggestive of achylia. There may be no symptoms whatever or on the contrary the patient may complain markedly. The appetite may be reduced, there may be a marked aversion to meat, fullness of stomach, gas, or occasional paroxysm of pain may occur. These attacks may occur after eating, soon after a meal, soon disappearing or persisting for a considerable period of time. Vomiting occasionally occurs but it is not a constant symptom. It may occur soon after eating and consist of coarse, undigested remnants of food, usually without blood. Headache and vertigo are common. The bowels are often sluggish but diarrhea is common in the later stages and is a characteristic symptom.

Einhorn has called attention to the fact that there are occasional cases of achylia that present symptoms quite parallel to those of hyperacidity. The only way to arrive at a diagnosis is by gastric analysis, which may have to be repeated. William Hunter stated that pernicious anemia should be laid at the door of mouth infection. Pernicious anemia has also been ascribed to some general disturbance of the lipid functions of the body with a production of abnormal hemolytic substances liberated in undue amounts exerting a destructive influence on the red blood cells.

CASE I.—T. E. H.; age forty-eight; married; merchant; American; first seen in August, 1916.

*Patient's Complaint:* Comes for examination because of belching after food, gas, vomiting spells, and sick headaches.

*Family History:* Analysis of the family history does not reveal anything pertinent to the present illness.

*Previous History:* There have been several attacks of tonsillitis at various periods in his early life. Tonsils were not removed but were submerged and shrunken at the time when

he was first seen. Patient has had pains (rheumatic?) in the knee joints, hips, and shoulders at various intervals throughout the past ten years. These pains were not severe enough to put him in bed but were troublesome and inconvenienced him a great deal. There was a Neisser infection at the age of 24. Patient was treated for typhoid fever four years before he was seen by me. Denies any syphilis.

*Habits:* Smokes two cigars daily. Does not use cigarettes or pipe. Alcohol is used sparingly. Patient states he is not a heavy eater.

*Present Disease:* The date of the onset of the present disease is not definite. The belching and gas for which he comes have been coming on more or less gradually and increasing in severity throughout the past four or five years. These symptoms were not troublesome enough at the outset to make him pay much attention to them and they were disregarded more or less. During the past year they have been troublesome enough, especially the belching and vomiting, to make him seek relief from a physician. The patient is troubled also with what he calls "canker sores" in his mouth for the past few weeks. These have also been present at a previous period some months ago. Analysis of the gastrointestinal symptom reveals that his belching and eructations have a rather definite relationship to his eating and are much worse after such foods as fatty substances, fried meats, or gravies. Tea is not well tolerated. There is no definite pain at any time in the abdomen. No jaundice. The appetite is much disturbed and has been so for five or six years back. There are no symptoms referred to the heart or circulatory system. There is no particular trouble with urination excepting that occasionally and at long intervals there is a little difficulty in urination due to an old stricture. Dizziness is an occasional complaint. There has been no appreciable loss of weight.

*Examination of the Patient:* There is nothing abnormal with respect to the temperature, pulse or respiration. The patient is a medium sized individual, weight 150 (usual 160); the skin has a rather sallow color with perhaps a tinge of yellow; there is no abnormality with respect to the bones, joints, or lymph glands; station and gait are normal; patient is a bit listless but there is no other mental abnormality; there are no skin lesions, no scars, no edema, and no external ulceration. Examination of the throat shows the tonsils rather shrunken and submerged but without visible pus. There are rather numerous small ulcer areas in the mouth the size of a pinhead or a little larger. The teeth looked good but were later found to be abscessed. There is no apparent trouble in the sinuses, nares, or ears. The pupils are equal on the two sides, react to light and in accommodation. The thyroid is not enlarged. The thorax is normal in appearance, and with respect to equality of respiration on the two sides. No abnormal findings can be determined with respect to the lungs, heart, or arterial system. Blood pressure was rather low, 108/60. The abdomen did not show anything unusual on inspection. Palpation was not tender and did not reveal any rigidity, mass, or abnormal splashing sounds. The liver, spleen, and gall bladder could not be palpated. The appendix region was not tender. There were no hemorrhoids. The genitourinary apparatus showed an old gonorrheal stricture. There were no scars on the genitalia indicating any specific lesions. Examination of the nervous system showed the patellar reflexes to be present on both sides; no Babinski; no Romberg; no ataxia; no nystagmus; sensation was normal with respect to touch and pain.

*Laboratory Findings:* The urine contained a few pus cells, possibly from the old stricture formation, specific gravity 1022, no albumin, no sugar, no blood, acid reaction. The blood examination showed red blood cell count 4,600,000; hemoglobin 90 per cent; white count 9,600; stained specimen showed the following differential count: Polymorphonuclears 68 per cent, small lymphocytes 20 per cent, large mononuclears 9 per cent, eosinophiles 2 per cent, basophiles 1 per cent; no parasites were seen; the red blood cells were well formed; there was no inequality in the size of the erythrocytes; no polychromasia; and no poikilocytosis; the blood Wassermann was negative. The stomach contents removed after an Ewald test breakfast showed no HCl to congo red after 20, 40, or 60 minutes. The total HCl was not more than 2.6 at any time. Lactic acid was absent. The gastric contents showed no blood and no sign of any unusual residue. No sarcinae or Oppler-Boas bacilli.

Two years following the time when this patient was first seen (February, 1918) the blood assumed changes sufficiently marked to enable a diagnosis of pernicious anemia to be made. The red blood cell count gradually decreased, reaching 1,200,000 as its lowest point; the white cells varied from 3,000 to 6,500; the hemoglobin progressively dropped and reached as low as 24 per cent (Dare); the color index was usually over 1 plus. The stained blood smears showed at various times the findings characteristic of pernicious anemia. There was anisocytosis, polychromasia, poikilocytosis, and nucleated red blood cells. This patient was subjected to repeated blood transfusions, medicinal treatment, all the teeth were removed while he was under the care of another physician, and despite everything that could be done the patient died in May, 1921.

**CASE II.**—Mrs. R. J. S.; age forty-nine; married; housewife; American; patient first seen in July, 1917, on account of persistent soreness in the mouth and gastric distress.

*Family History:* The parents are both living in good health. One sister died of pulmonary tuberculosis. No other condition determined having any apparent connection with the present illness.

*Previous History:* Patient had measles and whooping cough when a child. Since the age of twenty-four has had a great deal of trouble with teeth. Several were extracted on account of pyorrhea and those that remained were more or less involved with pyorrhea. She had refused to have all her teeth extracted prior to this time. At the age of thirty-five there was a moderate attack of rheumatism in the right knee with some swelling and redness, keeping patient in bed for several weeks but gradually subsiding. At other times during the past six or seven years there have been vague and more or less obscure pains in both shoulders and the knees, doubtless evidences of a chronic arthritic condition. No venereal disease that could be determined on examination.

*Present Illness:* Has had a rather insidious onset. For a number of years back she has complained of sores in the mouth coming as small, isolated, very painful ulcers. These would usually heal after a few days with the application of some home remedy. While these sores were present patient would refrain from eating to a considerable extent on account of the pain produced, so that there was more or less loss of weight. The highest weight which the patient reached has been 150 and has gradually declined to 127. Associated with this condition in the past two or three years there has been distress in the stomach region following the use of heavy foods. Could eat soft foods, such as eggs, cereals, and liquids without any distress, but burning in the stomach region and a feeling of weight and discomfort followed the taking of heavier foods more or less constantly. Patient was not able to ascribe the appearance of this trouble to any definite condition as far as she knew. It was not constant and might disappear for relatively short periods. There were no symptoms referable directly to the heart, lungs, or urinary system.

*Physical Examination:* Shows a rather emaciated woman, weight 127, slender frame. There were no palpable lymph glands. The joints were not enlarged, tender or deformed. Movement of the right shoulder and left knee showed distinct crepitation. The skin did not show any lesions or scars. Examination of the mouth showed two or three small, clear-cut, punched out, ulcerated lesions not larger than a grain of wheat. These were surrounded by a small area of reddening; the lesions were very painful to touch. The gums were in bad condition and pus could be pressed out from around several of the teeth. The thorax was well shaped and no disease could be determined with respect to the heart and lungs. The abdomen was retracted and did not show anything unusual on inspection. Dullness over the liver and spleen was not increased and these structures could not be felt on palpation. There was no unusual tenderness over the epigastrium even during the periods when the patient complained of distress here. No increased visible peristalsis. No disease could be determined in the gall bladder or appendix by the usual methods of examination. No disease was found with respect to the genitourinary apparatus.

*Laboratory Findings:* The urine showed a normal amount secreted in twenty-four hours, specific gravity 1018, acid, no albumin, no sugar, no casts, or other abnormal cellular



elements in the sediment. The blood findings showed red blood cells 4,220,000; white cells 7,600; hemoglobin 92 per cent; differential count: Polymorphonuclear 69 per cent, small lymphocytes 19 per cent, large mononuclear 9 per cent, eosinophiles 2 per cent, mast cells 1 per cent. The gastric contents were removed forty minutes after an Ewald test breakfast and showed no free HCl, total HCl 6, no blood, no lactic acid, and no apparent residue in the stomach.

A diagnosis was made of achylia gastrica and the patient put upon large doses of dilute HCl. She showed marked improvement with respect to the gastric distress following heavy foods and also a prompt improvement in the appearance of the ulcerations in the mouth. At this time the patient refused to have all her teeth extracted, but did visit a dentist and had several of the worst ones removed.

The patient did not gain in weight and was not seen for a period of several months while she remained fairly well. Early in 1918 the patient showed distinct evidences of pernicious anemia with a red count of 1,640,000; white count 3,100; hemoglobin 32 per cent (Dare); color index .99 plus. The stained blood smear showed marked distortion and deformity of the red blood cells, marked inequality of size varying from large macrocytes to very small microcytes. Many of the red blood cells showed nuclei. There was present also at this same time distinct evidences of involvement of the spinal cord. There was moderate ataxia, marked paresthesia around the trunk and back, constricting pains around the abdomen, and pains in the shoulder. The patellars were 1 plus; no Babinski; Romberg 2 plus; pupils equal on two sides and react promptly to light. The blood Wassermann at this time was negative (twice). The spinal fluid examination showed a clear fluid, 3 cells per c.mm., globulin not increased, no organisms seen in the stained smear, spinal fluid Wassermann negative in amounts up to 1.5 c.c.

At this time the patient consented to have all her teeth removed showing large amounts of pus at the roots. The anemia persisted despite the ordinary methods of treatment, including two citrated blood transfusions of 500 c.c. each. The patient died after three months with evidences of very advanced anemia with marked cord involvement.

CASE III.—L. F.; age fifty-six; married; merchant; first seen June, 1916.

*Patient's Complaint:* When this patient was first seen, his chief complaint was distress and pain in the upper abdomen between the epigastrium and the umbilicus. This pain usually followed the taking of food but there was no constant relationship. There was no relationship to the amount of pain as light meals or light foods would be followed by distress in the same way as heavy meals.

*Family History:* There is nothing unusual in the family history which would have any bearing on the present trouble. The parents and the relatives, in the main, have reached advanced ages.

*Previous History:* There is a very definite history of rheumatic pains or lumbago about twenty-five years ago. Has been confined to sanitariums at intervals for this condition. There is no definite history of attacks of tonsillitis but has complained frequently of sore throat. The tonsils have never been removed. There is no history of any venereal disease. Patient has always worked fairly hard in his business and has eaten fairly well. Alcohol is used moderately, also six or seven cigars daily.

*Present Complaint:* Dates back two or three years. It is not possible to place a precise or definite time limit on the beginning of his trouble because the onset is rather insidious. In addition to the digestive disturbances mentioned above, the patient has noticed a distinct sense of weakness or fatigue. He is not able to do his work well. Has to rest frequently and thinks that his efficiency is distinctly below par.

Examination at this time showed a moderately well developed man. Weight 154. Looks rather pale with a slightly pasty complexion. No enlarged glands could be palpated. The joints and bones did not show any definite disease to external examination. Skin rather sallow, no skin lesions or scars. The teeth showed a considerable amount of repair work and there are evidences at the gums of infection. The tonsils were somewhat submerged

but did not exude any pus on retraction of the pillars. The tongue was not unusually red. There are no ulcers in the mouth or evidence of stomatitis. Thyroid not large or palpable. The thorax is fair size showing a fairly equal expansion on the two sides. No disease was found with reference to the pulmonary system. The heart did not show anything unusual excepting a very soft systolic bruit heard in the fourth and fifth left interspace in the mid-clavicular line and not being transmitted well. The blood pressure is 135/60. The abdomen was rather retracted and did not show anything on inspection. The liver and spleen could not be palpated. Deep pressure did not elicit any pain over region of gall bladder or appendix. The rectal muscles were not rigid. There was no disease to be determined with respect to the genitourinary apparatus. The urine showed specific gravity 1.018, no albumin, no sugar, no blood, and one or two pale hyalin casts in the centrifuged specimen. The blood examination at this time (June, 1916) showed hemoglobin 88 per cent, red blood count 4,600,000, white count 6,500, differential count: Polymorphonuclears 72 per cent, small lymphocytes 21 per cent, eosinophiles 1 per cent, mast cells 1 per cent, large mononuclears 5 per cent. There was no evidence of any change in the size of the red blood cells no nucleated red blood cells seen, and no difference in the staining power. The gastric analysis showed at this time an entire absence of HCl both free and combined. Tests for pepsin were not made. There was no blood present and no mucus. The food left the stomach rather quicker than was normal.

At this time a diagnosis of achylia gastrica was made and the patient advised to have his abscessed teeth cared for. He lived out of the city and said this would be done. Patient was not seen again for a period of four years. He returned for examination in November, 1919. At this time the predominating complaint and trouble was referred to the blood. The clinical picture was that of a pernicious anemia. The hemoglobin was 48 per cent, red blood cell count 2,120,000, color index 1.1, the white count 4,550. Differential blood count was as follows: Polymorphonuclear 82 per cent, small lymphocytes 12 per cent, large mononuclears 5 per cent, eosinophiles 1 per cent, mast cells 0.

The stained specimen showed distinct evidences of polychromasia, anisocytosis, and poikilocytosis. The clinical picture was very definitely characteristic. The color of the skin was pale, sallow, and slightly yellow. There were sensory disturbances of both legs, feeling of prickling and numbness on the soles of the feet and calves of the legs. However, the reflexes were all retained in their normal way. Examination of the stomach contents at this time showed a persistence of the achylia gastrica with findings very similar to those of four years before. The condition of the teeth showed a large number of alveolar abscesses as revealed by the x-ray and tonsils definitely infected. The tongue was very red and there was a complaint of soreness or pain from occasional attacks of stomatitis.

The origin of pernicious anemia is not yet solved. However, many clinicians have definite opinions that such anemias have a connection or relationship with persisting infections wherever they may be localized. The fact that examination may not reveal such infections in particular instances is not a satisfactory reason for excluding them, in view of the great difficulty of determining obscure infectious processes in such inaccessible places as some of the deeper bony sinuses, the bile ducts, intestinal canal, pelvic organs, and other structures.

The work already done has convinced most clinicians that local infections, especially of the more pronounced types, can produce functional disturbances in practically any organ of the body. It is reasonable to assume that such long continued infections may produce an achylia gastrica with a persistently low gastric secretion such as was present in the three cases here cited. The presence of achylia gastrica in a patient showing a severe chronic focal infection should put one on his guard against a possible oncoming per-

pernicious anemia developing. It cannot be said that even the removal of all foci during the pre-anemic stage would prevent the development of a genuine pernicious anemia but it may be urged that the removal of such foci is highly desirable in the hope of preventing such a development. The association of achylia gastrica and pernicious anemia is so well known that these cases are cited only with the intent of calling attention to the sequence of focal infection—achylia gastrica—anemia in this respective order. If this chain can be broken even occasionally it means a human life saved, which is sufficient reason for the citations herein mentioned.

#### CONCLUSIONS

(1) Three cases are cited, in each of which patients having foci of severe infections passed through the stage of an achylia gastrica and later pernicious anemia.

(2) The assumption that long continued foci of infection may depress the gastric secretion and produce an achylia gastrica is justifiable.

(3) There is no reason to believe, on the basis of any evidence yet brought to bear, that achylia gastrica in itself is a predisposing cause of pernicious anemia.

(4) There is clinical evidence to believe that focal infections long continued may depress the function of the bone marrow and prevent the formation and development of new red blood cells.

(5) It is urged that when achylia gastrica is discovered in a patient showing evidences of chronic focal infections that the possibility of the development of pernicious anemia be carried in mind with the view of insisting that all infections be removed insofar as is humanly possible. Achylia gastrica is used here in the sense of a depression of the gastric secretion with relation to HCl and not necessarily with complete absence of gastric ferments.

## II. THE VALUE OF THE REFRACTO-VISCOSIMETRIC PROPERTIES OF THE BLOOD SERUM IN CANCER\*

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IN a previous paper studies recorded on the blood of syphilitic patients showed that there is a definite decrease in the refracto-viscosimetric quotient which is attributed to an increase in the serum-globulin. It was further demonstrated that treatment for syphilis had a marked influence on this quotient, with a tendency to bring it to normal.

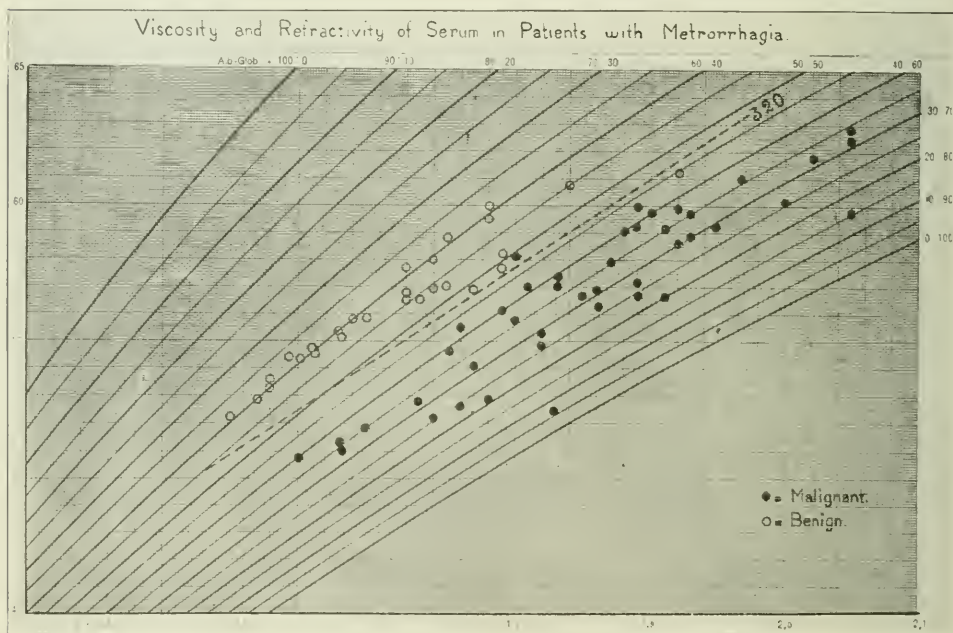


Fig. 1.

The examination of patients in the Mayo Clinic revealed a decreased quotient in various other diseases, such as cancer, tuberculosis, actinomycosis, and sarcoma. The findings in relation to cancer were selected for the present study.

The knowledge on this subject seems to be restricted, and though my study is in some ways a duplication of Loebner's work, it may be of value in confirming and correcting some of her findings. A discussion of some of the points in Loebner's conclusions will readily reveal the new aspect of the problem. Loebner calculated the amount of protein in the serum with the aid of

\*Received for publication, March 18, 1922.

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the refractometric index and the table of Reiss. I have not made this calculation as there are still some questionable points in the procedure. I have, however, confirmed Loebner's statement, that there is usually no decrease in the concentration of the serum of cancerous patients. The color of the serum in Loebner's cases was normal in twenty-one instances, darker than normal in twelve, and lighter in ten. In my series all possible shades from dark golden to light yellow were observed, which seems to indicate that the color is not of particular significance. Another point is of unusual interest. The refractivity and the viscosity of the serum were used in connection with the chart of Naegeli<sup>2</sup> to determine the albumin-globulin ratio. The most important conclusions are, that globulin is usually increased, that an improvement may be accompanied by a decrease in globulin and an unfavorable course by an increase. These conditions, however, were not absolutely constant.

TABLE I  
SERUM OF ONE HEALTHY PERSON EXAMINED OVER A PERIOD OF SIX MONTHS

TEST	EXPERIMENT	DATE	REFRACTIVITY	VISCOSITY	REFRACTO- VISCOSIMETRIC QUOTIENT	ALBUMIN	GLOBULIN
1	383	6/26	53.4	1.62	330	60	40
2	399	6/28	53.6	1.62	331	61	39
3	401	6/29	54.5	1.65	330	60	40
4	404	6/30	54.5	1.63	334	63	37
5	421	7/ 9	53.6	1.62	331	61	39
6	434	7/13	54.3	1.65	329	59	41
7	445	7/15	55.3	1.67	331	60	40
8	451	11/ 2	52.9	1.61	329	60	40
9	460	11/ 5	55.8	1.68	332	60	40
10	468	11/ 7	54.7	1.65	331	60	40
11	473	11/16	53.6	1.62	331	61	39
12	478	11/18	54.3	1.64	331	60	40
13	488	11/22	53.8	1.63	330	60	40
14	500	11/29	54.9	1.64	335	63	37

TABLE II  
SERUM OF WOMEN WHO HAD SYMPTOMS OF SEVERE METRORRHAGIA CAUSED BY BENIGN PELVIC TUMORS

TEST	EXPERIMENT	PATIENT	REFRACTIVITY	VISCOSITY	REFRACTO- VISCOSIMETRIC QUOTIENT	ALBUMIN	GLOBULIN
1	418	363948	58.0	1.74	333	59	41
2	374	361033	60.8	1.84	330	55	45
3	419	363537	56.9	1.74	327	54	46
4	446	375874	57.6	1.79	322	48	52
5	447	361040	55.8	1.69	330	58	42
6	450	163113	58.8	1.75	336	61	39
7	457	5256	61.1	1.92	318	44	56
8	458	243767	56.9	1.77	322	49	51
9	461	376368	58.2	1.79	326	51	49
10	465	376220	56.5	1.73	327	54	46
11	467	347728	56.9	1.75	325	52	48
12	470	363504	57.6	1.72	335	61	39
13	474	145680	55.3	1.67	331	60	40
14	475	377472	60.0	1.78	337	62	38
15	476	377138	56.7	1.73	328	57	43

The results given in Tables I to IV are evidence that globulin is increased in the serum of patients affected by a cancerous growth. This increase was found much more constantly than in Loebner's cases. Because of my previous criticism<sup>2</sup> it seemed advisable to compare the refractive and the viscosimetric findings without using the chart of Naegeli. Both of these physical properties depend largely on the protein content. A variation in the ratio of refractivity and viscosity is probably due to a change in the constituents of the protein or the serum as a whole. I cannot prove at this time the possibility that the variation depends entirely on the albumin-globulin ratio, and such an assumption is not even necessary with our present knowledge.

The refractive and the viscosimetric index of serum can be determined with great accuracy.<sup>2</sup> The quotient of refractivity and viscosity which has never been calculated in the form presented here, can be obtained as follows: For convenience the refractive index is expressed in Pulfrich's units.<sup>2</sup> These are multiplied by ten and divided by the viscosimetric index.

$$\text{Example: } \frac{56.0 \times 10}{1.75} = 320.$$

TABLE III

WOMEN WHO HAD SYMPTOMS OF METRORRHAGIA CAUSED BY CANCEROUS GROWTHS OF THE PELVIC ORGANS

TEST	EXPERIMENT	PATIENT	REFRACTIVITY	VISCOSITY	REFRACTO- VISCOSIMETRIC QUOTIENT	ALBUMIN	GLOBULIN
1	336	358916	58.0	1.87	310	36	64
2	340	358747	59.7	2.05	291	15	85
3	343	310843	59.3	1.95	304	29	71
4	353	349567	61.1	1.97	310	36	64
5	356	360742	62.5	2.05	305	32	68
6	407	334314	56.7	1.93	309	35	65
7	417	363839	55.6	1.76	316	43	57
8	420	361822	54.1	1.77	306	31	69
9	423	363880	53.8	1.73	311	38	62
10	428	361545	55.8	1.80	310	36	64
11	436	364539	56.9	1.86	306	31	69
12	438	362571	52.7	1.76	299	25	75
13	439	344862	54.9	1.82	301	26	74
14	442	363405	52.9	1.78	297	21	79
15	448	196042	60.0	1.89	317	43	57
16	449	375704	56.2	1.79	314	40	60
17	452	375314	61.8	2.02	306	32	68
18	453	374660	58.7	1.92	306	31	69
19	454	374790	56.7	1.85	306	32	68
20	455	376119	57.1	1.83	312	38	62
21	456	312183	57.1	1.89	302	26	74
22	459	139903	51.3	1.67	307	36	64
23	464	375722	60.0	1.94	309	35	65
24	466	375928	56.5	1.86	302	29	71
25	469	376627	51.8	1.69	306	35	65
26	471	376800	56.7	1.91	297	20	80
27	473	376005	58.2	1.80	323	49	51
28	477	377432	57.6	1.81	319	45	55
29	483	370087	60.4	2.08	290	15	85

## DISCUSSION

In Table I a basis is given for the study of physiologic and instrumental variations. The fact that changes in refractivity and viscosity are parallel to such an extent that the quotient is almost constant, eliminates great instrumental errors. Such errors would not affect both readings in the same sense. Under physiologic conditions the variation of the refractivity and of the viscosity is very slight. The refractivity varied from 52.9 to 55.3, and the viscosity from 1.61 to 1.67 with an average of 1.64. The average refracto-viscosimetric quotient is 331. The ratio of albumin to globulin as determined by Naegeli's chart shows an average value of from 60 to 40 and is within physiologic limits. This ratio cannot claim absolute validity because it is based on an indirect determination.

TABLE IV  
SERUM OF PATIENTS SUFFERING FROM VARIOUS FORMS OF CANCER

TEST	EXPERIMENT	PATIENT	LOCATION	REFRACTIVITY	VISCOSITY	REFRACTO-VISCOSIMETRIC QUOTIENT	ALBUMIN	GLOBULIN
1	331	358074	Abdomen	62.7	2.05	306	32	68
2	332	380465	Tongue	59.7	1.90	314	40	60
3	333	337562	Parotid gland	59.3	1.89	314	40	60
4	335	336595	Lip	54.7	1.75	312	40	60
5	367	356499	Lip	60.2	2.01	299	25	75
6	368	358192	Breast	59.3	1.91	310	36	64
7	369	358184	Rectum	52.4	1.83	287	6	94
8	393	360922	Stomach	52.2	1.74	300	25	75
9	405	322725	Rectum	59.8	1.64	310	40	60
10	406	362672	Parotid gland	58.9	1.93	305	30	70
11	410	362897	Penis	55.3	1.82	304	29	71
12	411	362475	Breast	57.1	1.81	316	42	58
13	415	362246	Stomach	51.1	1.67	306	35	65
14	416	362077	Breast	56.7	1.89	300	25	75
15	429	310127	Breast	57.3	1.83	313	40	60
16	440	329726	Bladder	59.1	1.88	314	40	60
17	329	359511	Vulva	57.1	1.85	309	35	65
18	375	—	Breast	56.9	1.81	315	40	60
19	431	331309	Breast	58.5	1.84	317	44	56
20	479	376037	Breast	58.2	1.85	315	40	60
21	480	377351	Breast	56.2	1.76	319	46	54
22	482	377938	Vagina	55.3	1.78	311	37	63
23	484	376159	Rectum	54.7	1.78	307	33	67
24	485	375819	Rectum	54.9	1.79	307	32	68
25	486	371442	Rectum	54.1	1.90	285	4	96

Table II demonstrates the fact that the blood of women with benign tumors is not affected. Refractivity and viscosity are within normal limits with one or two exceptions. The refracto-viscosimetric quotient ranges from 318 to 337, with an average of 328. The percentage of globulin is generally below 50 and rises slightly above it in three cases. It may be considered as normal.

Table III forms a certain contrast to Table II. All of the cases are malignant. The refractivity remains normal, while the viscosity of the serum is, as a rule, increased. Consequently the refractoviscosimetric quotient is de-

creased. The average is 307. The globulin percentage is decidedly increased, the average lies between 60 and 70.

In Table IV the findings of uterine cancer are generalized, showing that malignancy in any organ affects the refractoviscosimetric quotient in the same sense. It is markedly decreased.

#### CONCLUSION

The first series of experiments proves the validity of the technic and demonstrates the great constancy of the refractoviscosimetric quotient under normal conditions. The pathologic material gives evidence of marked change in the physical findings in the serum. While benign tumors do not affect the blood, any form of cancer causes a definite alteration. This can best be expressed by the refractoviscosimetric quotient which, in normal conditions, is almost always above 320, and in malignancy below 320, as shown in the chart.

#### ADDITIONAL POINTS IN THE TECHNIC OF THE DETERMINATION OF REFRACTIVITY AND VISCOSITY

1. In every paper dealing with the refractivity and viscosity of serum it has been emphasized that congestion of the vein before taking the blood sample modifies the result. In this study venous blood, obtained through a large needle, was used. The compression usually lasted only a few seconds, the blood flowed freely, and the first cubic centimeter was not taken. In order to determine whether or not congestion has a bad effect, blood was first withdrawn in the usual manner; a second sample was drawn after one minute, a third after two minutes, and a fourth after three minutes of intensive compression of the vein. The refractivity and the viscosity remained practically the same.

2. Samples of serum kept in the ice box for eight hours showed the same viscosity as those kept at room temperature.

3. The viscosity of the serum did not change if the serum was left with the blood clot for eight hours.

4. Serum obtained by centrifuging the sample of fresh blood showed the same viscosity as serum collected after the spontaneous retraction of the blood clot.

5. From a number of persons two blood samples were taken, in one of which slight hemolysis was produced. It was found that slight hemolysis does not alter the viscosity of the serum.

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# LABORATORY METHODS

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## SUGGESTED ADAPTATION TO THE GASOMETER METHOD OF THE DETERMINATION OF THE BASAL METABOLIC RATE FROM CARBON-DIOXIDE ELIMINATION\*

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**D**ETERMINATION of the basal metabolism has become a common procedure and many methods of indirect calorimetry have been developed. In the Tissot gasometer method, described fully by Boothby and Sandiford,<sup>1</sup> the patient inhales fresh air, the exhaled air being analyzed for  $O_2$  and  $CO_2$  content. The closed system described by Benedict<sup>2</sup> affords estimation of  $O_2$  absorbed by volume, and  $CO_2$  produced by weight. Other closed methods which are in most common use today deal with the  $O_2$  consumption alone, assuming a respiratory quotient of .82 as described by Jones,<sup>3</sup> and by Benedict and Collins.<sup>4</sup>

In the routine use of the Tissot method with analysis of air by means of the Haldane apparatus, certain technical difficulties are found. These, however, are not in the collection of the exhaled air in the gasometer, which with properly working valves in the air conducting system gives satisfactory results. It is the air analysis, particularly the determination of the oxygen content, that involves the greatest technical difficulty. The estimation of the  $CO_2$  content of a sample of exhaled air yields quite constant and accurate percentages which are easily checked. The estimation of  $O_2$  by absorption in pyrogallie acid is a considerably longer process. The variability in rate of absorption with the increased time necessary makes the process more subject to error. Experience with new technicians convinces one that to obtain accurate  $O_2$  estimation by this method requires several months' training.

For some months it has been noted that approximation of the basal metabolic rate could be obtained by finding the  $CO_2$  content of the exhaled air and using an average total  $CO_2$  plus  $O_2$  content. A series of 18 cases is found in Table I in which the average results obtained when a constant figure was used for total  $CO_2$  plus  $O_2$  with estimation of  $CO_2$  only, are compared with the results obtained when the individual values of  $O_2$  as well as  $CO_2$  were determined. The variation in the results obtained is in no individual case greater than 7 per cent and in 18 cases the average difference is 3.5 per cent. Such variation was not sufficient to alter the clinical interpretation of the values obtained in any case.

Recently King<sup>5</sup> has studied protocols of two groups of experiments with the Atwater Chamber Calorimeter by Benedict and Carpenter, and by Sonder-

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Received for publication, Dec. 3, 1921.

TABLE I

Comparison of values obtained for the basal metabolic rate

(a) when the amounts of  $O_2$  and  $CO_2$  produced are obtained for each patient,(b) when the  $CO_2$  produced only is obtained and an average total  $CO_2$  plus  $O_2$  figure is used.

CASE NUMBER	(A)	(B)	CASE NUMBER	(A)	(B)
1	+34	+29	10	+19	+16
2	+27	+25	11	+ 2	- 3
3	-12	-13	12	+ 4	- 2
4	- 2	- 5	13	+93	+97
5	+24	+27	14	+ 2	- 5
6	+59	+54	15	+ 4	- 2
7	+ 1	0	16	+17	+12
8	- 4	- 7	17	0	+ 1
9	-15	-16	18	+10	+ 4

strom, Meyer and Du Bois and has shown from their figures that there is a somewhat higher coefficient of correlation between  $CO_2$  and measured calories than between  $O_2$  and measured calories. He describes a method of determining the basal metabolic rate from the  $CO_2$  content of exhaled air, using the open method with collection of the  $CO_2$  in soda lime and estimation of its weight, and proposes a scale of normal figures\* representing grams of  $CO_2$  produced per hour per square meter of body surface, for men and women of different ages. King obtained this scale from his own findings in seventeen normal individuals averaged with the findings of Benedict, Emmes, Roth and Smith. King concludes that the  $CO_2$  elimination seems to be at least as accurate and possibly a more accurate index of heat production than the  $O_2$  consumption. In 1862 Pettenkofer<sup>6</sup> had constructed a respiration apparatus in which only the amount of  $CO_2$  produced was measured and Voit calculated the heat production in the body using heat values obtained in his laboratory for protein, fat and carbohydrate.

Besides the indirect approximation of the basal metabolism which, for a considerable time past, we have observed to be possible, as evidenced by the series of cases in Table I, the direct estimation of  $CO_2$  elimination with comparison to normal figures lends itself readily to the Tissot method and volumetric determination of the  $CO_2$  content. The advantages of the open method are evident, with its prevention of the danger of respiratory infection and the lessened disturbance to the patient with the open mask. The technical difficulty of determining  $CO_2$  volumetrically is scarcely, if any, greater than its determination by weight, and while correction must be made for temperature and barometric pressure, the use of scales is obviated.

To those who have the Tissot spirometer and do not have an expert air analyst, the estimation of  $CO_2$  volumetrically would seem to give more accurate information than  $O_2$  consumption figures obtained by other than thoroughly

\* $CO_2$  production in grams proposed by King for normal individuals.

Ages	Men	Women
15-20	14.03	12.75
20-30	12.98	11.95
30-40	12.86	11.85
40-50	12.52	11.74
50-60	12.21	11.37
60-70	11.86	11.05
70-80	11.53	10.71

experienced technicians:  $\text{CO}_2$  absorbs quite rapidly in the sodium-hydroxide solution and check readings are readily obtained. The apparatus necessary is simplified when the  $\text{CO}_2$  content only is determined since that portion of the Haldane apparatus used in the absorption of  $\text{O}_2$  in pyrogallie acid can be done away with. Besides simplification of apparatus there is shortening of computation.

The volumetric method has the added advantage of giving the total ventilation of the lungs for a certain definite period of time, which is not obtainable with the weight method of determining  $\text{CO}_2$ . A series of twenty-six cases is given in which the basal metabolic rate obtained from complete air analysis is compared with that obtained from  $\text{CO}_2$  produced. The variation in no case is greater than 6 and averages about 3 per cent. Results obtained check well with clinical diagnosis. The computation simply consists of taking the total ventilation, per hour, corrected to standard temperature and barometric pressure  $\times$  per cent  $\text{CO}_2 \times 1.96$  (to convert liters  $\text{CO}_2$  to grams)  $\div$  surface area (Dubois formula) with comparison to proposed normal figures for  $\text{CO}_2$  elimination.

In another series of thirty-three routine determinations with complete air analysis computations were made by assuming an average respiratory quotient of .82. The oxygen was then estimated by the simple ratio,  $\frac{\text{CO}_2 \text{ produced}}{\times (\text{oxygen absorbed})}$

TABLE II

Table II shows comparative values for the basal metabolic rate when obtained from the  $\text{CO}_2$  eliminated and when obtained by the estimation of both  $\text{O}_2$  absorbed and  $\text{CO}_2$  produced.

	NAME	AGE	BODY SURFACE DUBOIS FORMULA	$\text{CO}_2$ ELIMINATED PER HR. PER SQ. M. BODY SUR- FACE (GMS.)	PER CENT DEVI- ATION OF $\text{CO}_2$ PRODUCED FROM AVERAGE	THE BASAL METAB- OLISM PERCENTILE, USUAL $\text{O}_2$ ABSORP- TION AND $\text{CO}_2$ ELIM- INATION ESTIMA- TION
1	S.E.B.	24	1.5	13.96	+16	+13
2	M.R.B.	29	1.56	17.53	+35	+30
3	H.O.B.	28	1.66	13.01	+ 8	+ 8
4	N.E.B.	52	1.43	11.40	+ 0.2	- 1
5	J.A.B.	27	1.73	18.08	+39	+40
6	J.A.B.	27	1.76	11.71	-10	-9
7	S.E.B.	24	1.5	13.41	+12	+ 9
8	H.O.B.	28	1.66	13.33	+11	+ 9
9	A.N.B.	46	1.71	11.80	+ 0.5	0
10	E.D.B.	16	2.34	12.18	- 4	-10
11	A.U.B.	30	2.00	11.75	- 9	-11
12	L.A.B.	23	1.62	12.51	+ 4	0
13	S.B.	23	1.72	12.90	+ 4	+2
14	S.B.	23	1.73	10.48	-12	-15
15	C.B.	37	2.2	11.78	- 0.5	- 2
16	M.W.	28	1.345	15.51	+29	+27
17	W.H.A.	33	1.87	11.51	-10	-12
18	R.A.A.	29	1.84	12.77	- 2	- 2
19	A.A.	26	1.51	18.88	+58	+59
20	N.A.	31	1.63	10.88	- 8	- 8
21	G.A.	42	2.08	10.79	- 8	-10
22	M.A.	29	1.68	13.27	+ 2	+ 1
23	M.A.	52	1.76	11.05	-9	- 8
24	H.B.A.	48	1.615	13.39	+14	+10
25	V.A.	24	1.52	13.76	+15	+17
26	L.A.	23	1.86	13.88	+ 6	0

= .82, the  $\text{CO}_2$  value only being actually determined. The values thus obtained were compared to those from complete air analysis in the same cases and to those from  $\text{CO}_2$  production in grams. The average variation from complete air analysis in the computed basal metabolism was 4.8 per cent, from  $\text{CO}_2$  production in grams 3.4 per cent. A maximum variation in a diabetic with a respiratory quotient of .70 was 14 per cent in the former instance and 12 per cent in the latter. It is apparent that estimation of the basal metabolism from  $\text{CO}_2$  production is not applicable in pathological conditions in which the respiratory quotient is greatly altered. In the series studied, computation with an assumed respiratory quotient gave only a slightly greater variation than the  $\text{CO}_2$  production in grams from the basal metabolism values of complete air analysis.

In diabetes with its altered respiratory quotient and in acidosis,  $\text{CO}_2$  elimination has a limited value only. Certain cases of obesity have also shown an altered respiratory quotient and in this condition estimation of  $\text{CO}_2$  elimination may be of limited value.  $\text{CO}_2$  produced can be readily determined volumetrically with usual care and, in small clinics and laboratories where an experienced air analyst is not available, may be used as a relatively accurate index to the basal metabolism.

#### CONCLUSIONS

1. Adaptation of the  $\text{CO}_2$  determination of the basal metabolism to the Tissot spirometer is suggested.
2. This method has the advantage of all open methods, with less danger of infection and less disturbance to the patient.
3. The total pulmonary ventilation is obtained with the volumetric determination of the  $\text{CO}_2$  which is not possible with the weight method. The method is simple and results can be readily checked.
4. Results obtained by this method in a series of 26 cases give satisfactory clinical evidence and check well with those obtained by complete air analysis. In another series of thirty-three routine determinations comparison of results from  $\text{CO}_2$  production in grams to complete air analysis showed a maximum variation of 12 per cent in a diabetic with a low respiratory quotient. In that condition satisfactory checks with complete air analysis were not obtained.

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## THE CLINICAL VALUE OF BASAL METABOLISM DETERMINATION\*

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THIS paper briefly reviews the literature on the diagnostic value of the test, reports illustrative cases, with notes on the method of the test.

Of the calories kinetic in the food each day the part expended dynamically in work varies with the individual and with the task, but the part that is expended in keeping the vital processes going, that keeps the vital machine running, has been found not variable in the individual, and in different individuals has been found so closely proportional to height, weight, sex, and age that the normal can be predicted with negligible error. This necessary minimum of activity is the basal metabolism. Studies in diseased conditions, exclusive of fevers all of which give an increased rate, have shown the most definite groups with abnormal basal metabolism to be those cases with disturbances in the activity of the thyroid gland. McCaskey says "while recognizing the paramount position of the general clinical judgment in every case, variations in the basal metabolic rate as indirectly determined by oxygen consumption must be regarded as the most scientific and practical index of thyroid 'toxicity.'"<sup>1</sup> Boothby states that at least 95 per cent of all abnormally increased rates are due to hyperthyroidism if there is no fever.<sup>2</sup>

Increased basal metabolic rates are found in hyperthyroidism, in all febrile conditions, and in the active state of acromegaly<sup>3</sup>. In other diseases as essential hypertension, pernicious anemia, leukemia, diabetics with acidosis, cases with dyspnea, a rate above normal may be present occasionally. A decreased basal metabolism is always present in myxedema, in cretins, and in a lesser degree in hypopituitarism; the rate is low in inanition, starvation, and undernourished diabetics. Excluding these few other conditions changes in the basal metabolic rate are due to thyroid disease. The determination of basal metabolism in thyroid disease is like the use of the thermometer in febrile diseases and answers the same purpose of diagnosis, measuring the severity, marking the course of the disease and the effect of treatment.

A routine estimation of the basal metabolism should be made (1) in cases with goiter to ascertain possible toxicity; (2) in cases with or without goiter having symptoms resembling those caused by hyperthyroidism; (3) in similar cases with symptoms of thyroid deficiency; (4) in cases of obesity to differentiate those due to thyroid and pituitary disease<sup>4</sup>; (5) for an accurate measure of the effect of treatment of thyroid disease.

The diagnostic value of the basal rate is greatest in the group of cases with one or more of the symptoms of those caused by hyperthyroidism. Cardiac disturbances as tachycardia, cardiac myasthenia, and palpitation; fine

\*Received for publication, Dec. 12, 1921.

tremors, general debility, loss of weight, anemia, attacks of vomiting and diarrhea, psychic changes as depression and irritability, psychasthenia, sweats, are symptoms obviously of the most diverse etiology, but are symptoms which the exclusion of thyroid disturbance or its acceptance as cause can be made by a determination of the basal metabolism "as the final court of appeal."

<sup>5</sup>This test is especially useful in the differential diagnosis of hysteria, neurasthenia, early tuberculosis, and neuroses simulating thyroid disease. It is necessary in the diagnosis of "effort syndrome." Da Costa's irritable heart of soldiers, in which there is an increased sensitiveness to epinephrin but a normal basal rate. There has been needless confusion due to the exploitation of the epinephrin reaction. Epinephrin raises the basal metabolism but no relation has been found between the amount of the rise and the intensity of a hyperthyroidism, its effect being attributed to action on the sympathetic nervous system: "there is no evidence that the group of neurasthenics with a positive epinephrin reaction and a normal basal metabolism is dependent on variations in the secretion of the thyroid gland."<sup>6</sup> Likewise effort syndrome may be confused with the hyperthyroidism caused or brought out by the strain of service life in young men, in whom the thyroid enlargement of adolescence is not uncommon. The value of basal metabolism estimation in conditions at the time of the change of life in men as well as in women, when glandular balances are upset, is a field for interesting work. The rôle of the thyroid at this time of life is suggested by the inclusion of thyroid extract in the polyglandular treatment empirically used with clinical success. Metabolic work alone can determine the actual part of the thyroid in the phenomena of senescence.

DuBois in his paper on exophthalmic goiter says: "Exophthalmic goiter stands out as the disease of increased metabolism, and the increased metabolism stands out as the chief symptom of hyperthyroidism. The determination of heat production seems to afford the best index of the course and severity of the disease. There was great need for some purely objective test in hyperthyroidism to indicate the effect of treatment." Many of the other symptoms are simply the effect of the increased heat production, all are variable and difficult to measure. "The rapidity of the heart action is perhaps the best guide, but the heart is often affected by other conditions, and damage to the heart may outlast the other symptoms. The sugar tolerance test depends on other glands as well as the thyroid, and has wide limits even in health." "In contrast to the other symptoms an increased basal metabolism is found with great regularity in exophthalmic goiter and in severe cases reaches a level found in no other condition. On the other hand in cretinism and myxedema the metabolism is lower than in any other disease. The administration of thyroid extract particularly in myxedema, raises the heat production." As Plummer states, "the thyroid produces its effect through metabolic processes."<sup>8</sup>

The basal metabolic rate gives a precise measure for the selection of treatment and the determination of its effect if any in diseases of the thyroid. It affords a mathematical measure of comparison between different cases for systematic study. Means and Aub<sup>9</sup> state that the level of the basal metabolism

TABLE I  
CASES FOR DIAGNOSIS

CASE	SEX	AGE	HT. CM.	WT. KG.	OX. VOL.		CAL. P.		NORM. CAL.		% VAL.	DIAGNOSIS FROM TOXIC THYROID
					P. 10M.	2311	DAY	1618	BM PER D.	1730		
1	M	23	180	66.8							7-	Goiter, tremor, P. 60, sweats, "nervous," nontoxic adenoma plus neurosis
2	M	22	166	55.1	4617	1622			1506		8+	Neurosis, myocarditis, local tetany
3	M	21	171	49.6	1935	1355			1463		8-	Intention tremor, multiple sclerosis
4	F	39	166	58.3	1881	1295			1538		2-	Tachycardia, tremor; cardiac
5	M	31	180	78.6	9768	1938			1838		5+	Irritable heart
6	F	31	155	46.	1917	1342			1255		7+	Neurosis following several operations
7	M	23	184	74.5	2764	1958			1566		5-	P. 70, tachycardia on exercise, tremor, irritable heart.
8	F	29	163	53.5	1839	1288			1333		4-	Goiter, loss weight, "nervous," nontoxic
9	M	23	175	58.	2201	1517			1584		4+	P. 100, loss weight, nervous, valve, heart dis.
10	M	38	177	72.4	2579	1805			1691		6+	Aschoffia, alipia, impotence, psychasthenia
11	M	20	182	70.3	2608	1826			1910		4-	Goiter, tachycardia; cardiac
12	M	25	172	57.4	2267	1588			1518		2+	Mitral regurg., neurosis; prev. diag. thyrotoxicosis
DIAGNOSIS THYROTOXICOSIS												
13	M	20	187	74.6	2360	2352			1893		24+	P. 111, pulse press. 70, early ex. goit.
14	F	25	176	51.1	2176	1524			1353		13+	P. 96, goiter, hot sens., loss weight, dilat. heart, hyperthyroidism
15	M	26	172	50.5	2387	1671			1446		15+	Small goiter, tremor; hyperthy. and bte.
16	M	26	174	60.1	2510	1758			1588		11+	Goit., trem., loss wt., mild toxic goit., quinin. hbr., 2 mo., BM 34, no symp. well
17	M	27	171	67.4	2317	2322			1666		39+	P. 98, trem., palp., no ex.; toxic thy.
18	F	41	165	46.1	1965	1376			1209		14+	P. 88, palp., goit., nervous; mild toxic
19	M	20	173	76.5	2118	1849			1849		18+	P. 85, goiter, tremor; mild toxic
20	M	28	180	61.4	2666	1867			1619		15+	P. 70, goit., tachy.; hyperthy. and mitral
21	M	20	178	68.2	2976	2084			1760		18+	P. 73, goit., trem., palp.; mild toxic
22	M	21	176	61.	2895	2027			1623		25+	P. 75, trem., tachy., nervous, hyperthy.
23	F	33	157	56.4	3603	2496			1330		8+	P. 120, no goit., no exoph.; tremor, diarrhoea

Height in centimeters, weight without clothes in kilos, corrected volume of oxygen retained per 10 minutes, calories basal rate per day (observed), and normal prediction per 24 hours, basal metabolism, from Harris-Benedict tables.

is a better guide to the degree of thyroid lack in hypothyroidism than the clinical picture and that the proper dosage of thyroid is best measured by the effect on the metabolic rate. (They found an average of 3 to 4 grains per day of thyroid extract brought the rate to normal, and 1 or 2 grains would keep it there.)

In hyperthyroidism the operative risk should be gauged by this test judged in combination with the other symptoms, particularly the cardiac reserve, and the need or character of surgical or medical treatment considered on an objective basis as much as possible. Rates of 100 plus or over are dangerous, and should be reduced by absolute rest in bed. Rates of 75 plus, very severe, rest, hot water injections or ligations, should be considered preliminary to partial thyroidectomy. Lahey and Jordan<sup>10</sup> warn against primary thyroidectomy in most cases with a rate of over 35 plus. This is very conservative. There are a few atypical cases of hyperthyroidism with rates almost normal. Sandiford<sup>3</sup> had 22 patients with an average basal rate of 66 plus, pulse 123; after two ligations and rest in bed the rates averaged 46 plus, pulse 115. Three months later the rates averaged 39 plus, pulse 107, and at discharge after thyroidectomy the rates averaged 16 plus, pulse 89. The three month interval between ligation and partial thyroidectomy seems to be too long. The basal rate should be tested every two weeks, and the operation timed accordingly. Of the cases cited 14 had a rate at the time of operation as high as before or higher. In exophthalmic goiter a second or third partial thyroidectomy may be indicated, or the x-ray may be used to bring metabolism to normal.

The Roentgen ray treatment has been compared with surgical results by Means and Aub.<sup>11</sup> "In the third year x-ray and surgery were alike," all patients were leading normal lives, rates averaged 13 plus, for both, the x-ray gave equally good results and in cases of equal toxicity there was less danger. They suggest using x-rays first, a cure may be effected, at least the metabolic rate will be reduced and surgery made safer. If the basal rate is high after surgery the x-ray may be employed again. Means' latest report is still more favorable to the x-ray in that the patients treated by the rays were normally active during the course of treatment.<sup>12</sup> Occasionally a case will not react favorably to the rays. Some surgeons object to the increased difficulty of the operation from fibrosis. DuBois says a partial thyroidectomy has been and perhaps always will be standard treatment.

The cases selected for Table I were referred for diagnosis, based on the metabolic rate.

Both groups in Table I had about the same clinical appearance, with palpitation, tremors, goiter, "nervousness"; the diagnosis was made on the basal metabolism, one group of cases largely cardiac, the other thyrotoxicoses. The male patients in both groups were service men in the Navy and from the War Risk Insurance Office, referred by Drs. Reed, Derbyshire and McDowell. Case 14 referred by Dr. Churchill, and 16, from Dr. Reed, are examples of possessing a low basal rate, scarcely above normal (13+ and 11+) with mild toxic symptoms; 23, referred by Dr. Churchill, without goiter or exophthalmos, had a basal rate of 88 plus, tremor, diarrhea, a severely toxic case.



TABLE II  
CASES SHOWING EFFECT OF REST

CASE	SEX	AGE	HT. CM.	WT. KG.	ON VOL. P. 10ML.	CAL. P. DAY	NOI. CAL. BAL PER D.	% VAR.	
1	F	36	166	44.1	2985	2976	1197	73+	Dec. 3, 1920, p. 132, severe exoph. goiter
				46.8	2929	1360	1224	27+	March 22, 1921, p. 96, absolute rest in bed
2	M	30	178	57.4	2981	2987	1551	53+	Oct. 25, 1920, p. 79, thyrotoxicosis
				57	2710	1898	1545	23+	Dec. 31, rest on ranch
				58.7	2604	1823	1569	16+	March 3, 1921, on ranch, light work, improved
3	M	41	176	56.5	2488	1742	1531	14+	June 21, seven x-ray treatments since March
				48.6	2399	2362	1352	74+	Oct. 6, 1920, p. 108, exoph. goiter
				53.8	3081	2157	1389	55+	Oct. 12, 45 gland removed, Dr. McDowell
				58.1	3408	2386	1498	65+	Dec. 24, p. 100 irreg., very nervous
4	M	24	188	37.	3629	2741	1423	77+	March 7, 1921, p. 100 irreg., very toxic
				Still classic ex. goiter, severe myocarditis, inoperable					June 24, p. 100 irreg., getting x-ray treatment
5	F	33	156	76.9	4131	2892	1903	52+	April 28, toxic adenoma
				76.8	3430	2402	1902	26+	June 23, rest only
				52.2	1797	1258	1288	3-	Tremor, palpitation, p. 118, Two months
				Previous BM 151, quieted by rest only.					Op. Dr. C. M. Fox, toxic adenoma.

Case 3 Table II in spite of a 4 5 thyroidectomy had a return of the full toxicity and with this a damage to the myocardium that makes the outlook discouraging. Rest was without effect, in his case, at present he is getting x-ray treatments. Rest in the last two cases brought the basal rate down to where the operative risk is minimal. Case 2 reached the limit of improvement for him with rest at 16 plus, but symptoms were more severe than the rate indicates. The roentgen treatments are causing a steady return to normal.

Case 1, Table III, was brought from 35 plus to 10 plus by rest alone with corresponding amelioration of the hyperthyroidism as regards other symptoms. A month later the rate was 25 plus, weekly x-ray treatments improved symptoms and reduced the rate. Case 2 referred by Dr. L. C. Kinney, had hyperthyroidism and a functional heart disturbance, he treated the patient with x-rays resulting in a symptomatic cure, rate 1 minus, the patient returned to college. Case 3 also given roentgen treatments by Dr. Kinney shows a gradual improvement under adverse circumstances. He had had a partial thyroidectomy in 1918, but in Oct., 1920, had a return of his goiter, a severe exophthalmic, and was studying in a business college. Dr. Kinney increased the dose of rays in Feb., 1921, when the basal rate had gone up to 30 plus again, and by July the patient was well except for the exophthalmos. Case 5 neglected to report for the metabolic test and the actuality of the effect of the rays on the thyroid was demonstrated in a rate of 36 minus, patient weak, she could hardly get about. Treatment was discontinued and the thyroid returned to normal, patient well. Case 6 referred by Dr. Fox had symptoms rather more toxic than the rate of 10 plus, accepted as the normal limit, indicated. X-ray treatments reduced the rate, relieved the nervousness and tachycardia, and as shown there was a gain of 3.7 kg. Case 7 was referred to Dr. Kinney for x-rays as hyperthyroidism. The basal rate in spite of hyperthyroid symptoms, was 19 minus. Thyroid extract increased the toxicity, so on the diagnosis of dysthyroidism Dr. Kinney gave irritating doses of the roentgen ray, with symptomatic cure, normal basal rate, and gain in weight.

Operative results followed have shown about the same results as reported by others; a complete cure in the case of toxic adenomas, and often discouraging immediate results in the exophthalmic goiters. Case LH, rate 63 plus, pulse 130-160 was practically unaffected by x-rays. Operation by Drs. Oatman and Rees, 2 3 thyroidectomy, brought the rate to 15 plus, pulse 80, she had had a severe exophthalmic goiter. Other cases have had recurrence. There are some cases that are almost uninfluenced by any method of handling. Three toxic adenomas, with rates of 13 plus, 45 plus, and 41 plus, operated on by Drs. McDowell, Fox, and Oatman respectively, are well, with rates after operation: 2 plus, 1 plus, and 5 plus.

Our experience agrees with that of others in emphasizing the importance of the general clinical observation as control of this objective test, the basal metabolism. This does not diminish the value of the information given by the basal rate, any more than clinical examination lessens the necessity of the routine use of the thermometer or the leucocyte count. It is the best guide in disturbance of the thyroid secretion. Generally speaking patients with hyper-

TABLE III

CASE	SEX	AGE	HT. CM.	WT. KG.	OX. VOL. P. 10ML.	CAL. P. DAY	NOE. CAL. BM PER D	% VAR.	X-RAY TREATMENT
1	M	30	164	56.9	2838	1987	1407	55+	May 18, goiter, tremor, p. 90, no exoph.
				55.2	2872	1501	1444	10+	June 10, rest, p. 70, pulse press. 60
				56.	2609	1826	1455	25+	July 20, rest and sol. brom., pulse 70
				54.6	2398	1679	1435	17+	Sept. 30, had 8 x-ray treatments, improved
2	M	22	174	51.4	2901	2050	1495	17+	Nov. 9, 1920, p. 104 hyperthy. and finet. heart
				60.2	2995	1601	1611	36+	June 11, 1921, weekly x-ray. Well, at school
3	M	24	182	68.3	3844	2088	1754	53+	Oct. 29, 1920, p. 155, severe exoph. goiter
				67.2	3471	2224	1740	23+	Dec. 1, 1920, 3 x-ray treatments per mo. In business college
				70.5	3006	2106	1784	17+	Jan. 11, 1921, 3 x-ray treatments per month. In business college
				71.4	3334	2234	1796	30+	Feb. 1, worse, 3 x-ray treatments per month. In business college
				72.4	2995	2096	1810	16+	March 8, improved. Weekly x-ray. In business college
				70.1	2930	2052	1778	15+	April 13, p. 75, weekly x-ray. In business college
				70.5	2974	2082	1784	16+	May 3, p. 78, weekly x-ray. In business college
				71.	2877	2014	1791	13+	June 16, p. 60, weekly x-ray. In business college
				71.	2491	1744	1791	3-	July 15, well except for exophthalmos
4	F	22	165	70.5	2589	1812	1784	1+	Aug. 5, p. 60
5	F	29	162	49.1	2339	1638	1281	28+	Three mo. previous BM was 67+
				46.4	2263	1655	1263	31+	Jan. 29, pulse 150, severe ex. goiter
				51.6	1203	843	1313	36-	June 6, p. 75, weak, x-ray discontinued
6	F	35	163	50.2	1741	1219	1299	10+	July 19, no treatment, well
				71.1	2144	1630	1473	10+	Nov. 12, 1920, hyperthyroidism, mild
				72.9	1987	1301	1485	6-	Jan. 21, x-ray treatment. No symptoms now
7	F	49	170	74.8	2125	1488	1503	1-	March 5, still well and gaining weight
				61.1	1322	1073	1324	19-	Feb. 13, dysthyroidism, p. 106, exophth
				59.5	2020	1415	1309	8+	June 14, x-ray treatment, pulse 80, improved
				62.5	1837	1286	1352	5-	Oct. 10, well. (Thy. ext. made worse, Feb.)

thyroidism with rates under 30 plus can be treated by roentgen rays as successfully as by surgical means, and the time needed for the raying is not excessive, as it is apt to be in more severe cases. The ray is invaluable as a follow-up when partial thyroidectomy has not entirely reduced the excessive secretion to normal. Surgery in severe cases must be cautious, but ligations must be followed by partial thyroidectomy before the establishment of collateral circulation, which means within three or four weeks. The basal rate should be measured and the operation done while the rate and pulse are yet declining. Frequent basal metabolism tests are necessary with present knowledge to determine the time of operation after preliminary rest in bed, and whether ligations or a primary thyroidectomy are safest, although the general clinical picture, with particular reference to the cardiac reserve, must be a controlling factor.

Case 1, Table IV, referred by Dr. Weir, had so low a basal metabolism, relieved by giving thyroid extract, that it is reasonable to suppose her toxic symptoms were due to a glandular unbalance associated with pregnancy. There was a lag between the restoration of a normal basal metabolism and her symptomatic relief, also present in Case 2. This woman had been suffering from the disturbances of the climacteric, weakness, irritability, fainting spells, and complained most of a paronychia that was painful and intractable, all symptoms aggravating in cycles of more or less four-week periods. The first basal rate was measured on an up-grade, 5-, the next during the depressed period was 17-. Feeding thyroid extract she feels better than she has for four years, the paronychia has not recurred in 5 months, she sleeps soundly and does her housework cheerfully. The rate is 26 plus, but her age is fifty, and the health is the proper guide as to what is normal for her, although her heart is being watched by her physician. Case 3 referred for metabolism by Dr. Winters is similar; menopause disturbances, premature in this case, associated with mucous colitis. She was given thyroid and ovarian extract, has gained weight, been relieved of her colitis, and sexually is returning to normal. The last two cases are included to guard against overenthusiasm. Case 4 has an eczema worse at menstruation, 5 is undergoing the menopause with considerable distress, palpitation tachycardia, suggestive of thyroid hypersecretion. Neither showed thyroid disturbance demonstrable by change in the basal metabolism.

#### TECHNIC

The method of making the estimation of basal metabolism is essential. The use of apparatus that has not the weight of approval of the men with the most experience might invalidate the work of those beginning to make the test, and tend to bring the test itself into discredit. The Tissot apparatus gives information that is necessary in the general investigation of metabolism, but to the clinician has no advantages to offer, while the requirement of a trained gas analyst and the factors for time and cost are insuperable obstacles outside of large supported clinics. On the other hand extreme portability is not needed, the patients too ill to come to the laboratory, as Bailey<sup>13</sup> says, are not the type of case in which basal metabolism determination is of value. The Benedict Portable Respiratory apparatus<sup>14</sup> seems to have all the simplicities possible to



TABLE IV  
CASES SHOWING ENDOCRINE UNBALANCE

CASE	SEX	AGE	HT. CM.	WT. KG.	OX. VOL. P. 10ML.	CAL. P. DAY	NOR. CAL. BAT PER D.	% VAR.	
1	F	26	165	51.1	1274	892	1327	53-	Feb. 3, preg. 4 mo., depressed, weak, persistent vomiting Feb. 8, taken thy. extr., same symp., by Feb. 10 felt well; 13M 9- on Feb. 19; remained well to term
				51.1	1908	1336	1327	0	
2	F	50	163	67.9	1719	1203	1372	5-	July 6, feeling fair, upgrade
				70.5	1667	1167	1397	17-	July 25, weak, fainting spells
				69.3	2000	1400	1386	1+	Aug. 1, thy. extr. daily, feels same
				67.4	1812	1268	1368	7-	Aug. 20, felt improved since 20th
				70.9	2736	1770	1401	26+	Oct. 25, feels better than for four years
3	F	34	156	44.1	1285	900	1207	25-	Aug. 22, mucous colitis, menopause
				44.5	1384	969	1211	20-	Oct. 4, thy. extr., improving
4	F	30	161	46.4	1768	1124	1229	9-	Nov. 4, thy., feels well, colitis gone
				70.2	2017	1412	1481	5-	Sept. 2, before menstruation
5	F	40	165	71.1	2043	1430	1493	3-	Sept. 13, after menstruation
				58.5	1963	1374	1333	3+	Menopause, palpitation, asthenia

careful work at present. It is portable in the sense of being movable about a hospital, but is best kept in a metabolism room to which patients may be brought on a litter if bed-ridden.

Roth<sup>15</sup> in a paper on the use of the Benedict apparatus says the chances of error lie chiefly in improper management of the subject. The patient should see the apparatus the day before the test, when it should be explained to him, lest fear of an unknown test, of suffocation, or excitement raise the oxygen consumption. He should come to the laboratory, or be brought there, in the morning, fasting, having had no food including tea or coffee since the previous evening. The metabolism room should be quiet, and no one allowed in during tests except the operator and nurse. The patient is placed in a recumbent position, with pillows as needed, in the darkened room, for half an hour before the test. The position must be comfortable so that movements, restlessness, or changes of position will not add to heat production, and the head must be high enough to allow the saliva to be swallowed easily with the mouthpiece in. When nervous tension or apprehension is unavoidable, after a short trial breathing into the apparatus the patient is best dismissed and the test made another day. Cases with severe thyrotoxicosis are unable to repress movements. Rarely it is impossible to get sufficient cooperation to make the test. The age of the patient to the nearest year, height without shoes in centimeters, weight to tenth kilo are noted, the weight to be taken at the time of the test, and the barometer is read. The temperature is taken to rule out fever.

Fill the spirometer three-fourths full of oxygen and start motor. Test for leaks. Place clamp on the patient's nose, have him try to breathe through the nose. Adjust the rubber mouthpiece attached to the three-way valve, inside the lips, outside the teeth, raising or lowering the support arm and changing the angle so it rests easily in the mouth. Tell the patient to breathe naturally and to avoid forced expiration. Cover his eyes with a light cloth. Turn the three-way valve at the end of an expiration. Read the temperature of the spirometer bell. Take patient's pulse. When the breathing has become regular, the excursions of the indicator showing a small loss at the end of each expiration, take readings as Emmes directs: Write the minute by the watch (the minute and second hands set together), then note the reading of the spirometer at the height of a normal expiration, immediately note the second by the watch, and write both beneath the figure for the minute. Repeat such readings several times a minute for two or three minutes, and again for two or three minutes just previous to the end of the test (10 min.). Take the pulse and respiration in the interval.

Subtracting the time readings, (the seconds conveniently written as decimal fractions of a minute) and the corresponding readings of the volume of oxygen in the spirometer taken at the start, from those taken at the end of the test, the number of c.c. of oxygen retained in a period of minutes is obtained, which may be computed as an average for ten minutes, averaged from the sum of the total number of readings, from both periods of the test. To reduce this volume to normal temperature and pressure of zero Centigrade and 760 mm. barometric pressure, this figure is multiplied by a factor from tables for the reduction of

gas volumes at different pressures and temperatures.<sup>16</sup> Should the temperature of the spirometer bell have risen during the period of the test add for each degree rise 1.8 c.c. to the observed volume before correction.

To obtain the basal heat production for 24 hours from the corrected volume of oxygen consumed per 10 minutes, this figure must be divided by 1000 to reduce to liters, multiplied by 6x24 (time factor) and by the caloric value of a liter of oxygen, 4.825 for an assumed respiratory quotient of 0.82, generally taken.  $6 \times 24 \times 1,000 \times 4.825 = 0.6948$ . 0.695 times the figure for the corrected volume of oxygen consumed in 10 minutes gives the basal heat production per 24 hours in calories.

If the respiratory quotient of 0.85 is assumed for the Portable apparatus as is done by the Nutrition Laboratory<sup>17</sup> the caloric value for the oxygen is 4.863 and the factor for the daily basal heat production from the c.c. oxygen per 10 minutes is 0.7003.

Having found the patient's basal metabolism in calories per day, there remains to determine the normal basal metabolism of the age, sex, height and weight of the patient. For this there are two methods of procedure. Both the Aub and DuBois normal standards, and the normal prediction tables of Harris and Benedict agree closely for adults, particularly between the ages of 20 and 40. On account of the small number of normal women over 40 measured, on which these analyses are based, the variation is greater for these older women. Both standards are derived from the same statistics. "For the pres-

REPORT OF BASAL METABOLISM DETERMINATION

PATIENT'S NAME				DATE		
ADDRESS				REF. BY DR.		
Average of readings of oxygen consumed per 10 minutes						
Period A		c.c.				
B		c.c.				
C		c.c. — average		c.c.		
add 1.8 c.c. for each degree rise in temperature						c.c.
Temperature spirometer bell,						
beginning	A	B	C	Barometer	mm.	
ending	A	B	C			
Factor to correct volume oxygen to 0 and 760 mm. 0.						
Corrected volume oxygen per 10 minutes				c.c.		
Calories per day, basal rate				calories.		
(RQ 0.85, factor 0.7003; RQ 0.82, factor 0.695.)						
Age	Height	cm.	Weight	lbs.	kg.	est. less
Harris-Benedict tables normal prediction:						
Factor, body weight and sex				—————(add)		
Factor, stature, age and sex				—————(add)		
Estimated calories per day (normal)						
Calories per day, observed, (above)						
Percentage variation from normal, basal metabolism						
Aub and DuBois standards:						
Body surface area, (height-weight chart) in sq. meters						
Calories per day per square meter, for age and sex				————— (multiply)		
Calories per day, basal heat production						
Calories observed, daily basal metabolism (above)						
Percentage variation from normal, basal metabolism						
Notes, cooperation and behavior						
Pulse	A	B	C	Respiration	A	B C temp.
Clinical notes						

ent persons who are presumably in good health are taken as normal individuals."<sup>18</sup> The standard for girls from 12 to 17 years old has been determined by Benedict and Hendry.<sup>19</sup> It averages 1250 calories per day irrespective of age, the accuracy is only slightly greater when weight is considered. These tables, as well as formulae for other children and for infants, are most conveniently found for reference in Carpenter's "Tables, Factors and Formulas for Computing Respiratory Exchange, etc.," which in small compass furnishes all the data needed for basal metabolism estimation.<sup>16</sup>

From the DuBois height-weight chart the body surface area in square meters is obtained, this figure multiplied by the average normal calories per square meter per day for the age and sex gives the normal 24-hour basal heat production. Comparison with the observed rate shows whether the latter is higher or lower, "plus" or "minus," and the percentage variation is the basal metabolic rate.

If the Harris-Benedict standard basal heat production is used the tables in "A Biometric Study of Basal Metabolism in Man"<sup>20</sup> (also given by Carpenter) give factors for the weight, and for the age and height, of the sexes, two figures which added give the figure for the calories of basal metabolism per day for the normal.

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- <sup>6</sup>Tompkins, Sturgis and Wearn: Studies on Epinephrin, II. Arch. Int. Med., Sept., 1919, xxiv, 267. Of the irritable heart cases many are also hypersensitive to epinephrin but show normal basal metabolism and no hyperthyroidism on clinical analysis. The metabolism always rises after epinephrin whether there is a reaction or not.
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## A NEW TYPE OF RECORDING SPIROMETER\*

BY R. BURTON-OPITZ, M.D., NEW YORK

THE type of spirometer which has been made use of by me in this laboratory, registers the volumes of the respiratory air as a continuous line upon the smoked paper of a kymograph. Moreover, since the action of its different parts may be reversed at any time, it may be employed to record a practically unlimited number of respiratory cycles. The resistance resident in its different parts is so slight that the subject of the experiment scarcely perceives any hindrance to the flow of the respiratory air. A very simple change in the adjustment of this apparatus enables the experimenter to register solely the volume of the tidal air or solely that of either the inspiratory or expiratory air.

The most essential part of this instrument is a cylinder which is placed horizontally and gives lodgment to a piston of hard rubber. The excursions of the latter are registered upon the smoked paper of a kymograph (Fig. 1). The cylinder (*C*) is made of brass and measures 30 cm. in length and 12 cm. in diameter. Its internal surface is ground absolutely true with pumice powder. Piston (*P*) is adjusted to move in a horizontal direction through this chamber without being able to form contact with the covers *D* and *D*<sub>1</sub>. In order to decrease its weight and to prevent all leakage of air, the thickness of the central area of its rubber disc has been reduced to about 0.5 cm., while the edges of this plate have been permitted to retain a breadth of 1 cm. This rubber disc is held in its proper position by a steel rod (*R*), measuring 2 mm. in diameter and 58 cm. in length. The ends of this rod rest in the central orifices of the covers of the cylinder. The internal surfaces of covers *D* and *D*<sub>1</sub> are equipped with three horizontal rods (*E*<sub>1</sub>), each of which measures 4 cm. in length. Their purpose is to restrict the lateral excursions of the piston, so that its surfaces cannot form contact with those of the covers. By this means, two dead spaces are formed in the ends of the cylinder which give lodgment to the orifices of two tubes, designated in the figure by the letters *B* and *B*<sub>1</sub>. A strong rod is fastened in a horizontal direction to the posterior surface of the cylinder. It serves as a means of adjusting the entire apparatus securely in the clamp of an upright stand (Fig. 2).

The aforesaid tubes *B* and *B*<sub>1</sub> possess an internal diameter of 2 cm. and an outside diameter of about 2.2 cm. They are curved and descend obliquely along the opposite sides of the cylinder to the lateral outlets *B* and *B*<sub>1</sub> of valve *V*. The latter consists of a capsule of brass which is fastened to the floor of the cylinder. It contains a rotating disc, measuring 7 cm. in diameter and 3 cm. in height. Two U-shaped tubes, possessing a diameter of 2 cm., pierce its mass in opposite directions to one another. The vertical axis *F* traversing

\*From the Physiological Laboratory of Columbia University.

Mr. Joseph Becker, 437 W. Fifty-ninth street, New York, has agreed to manufacture this instrument according to specifications.

Received for publication, January 17, 1922.

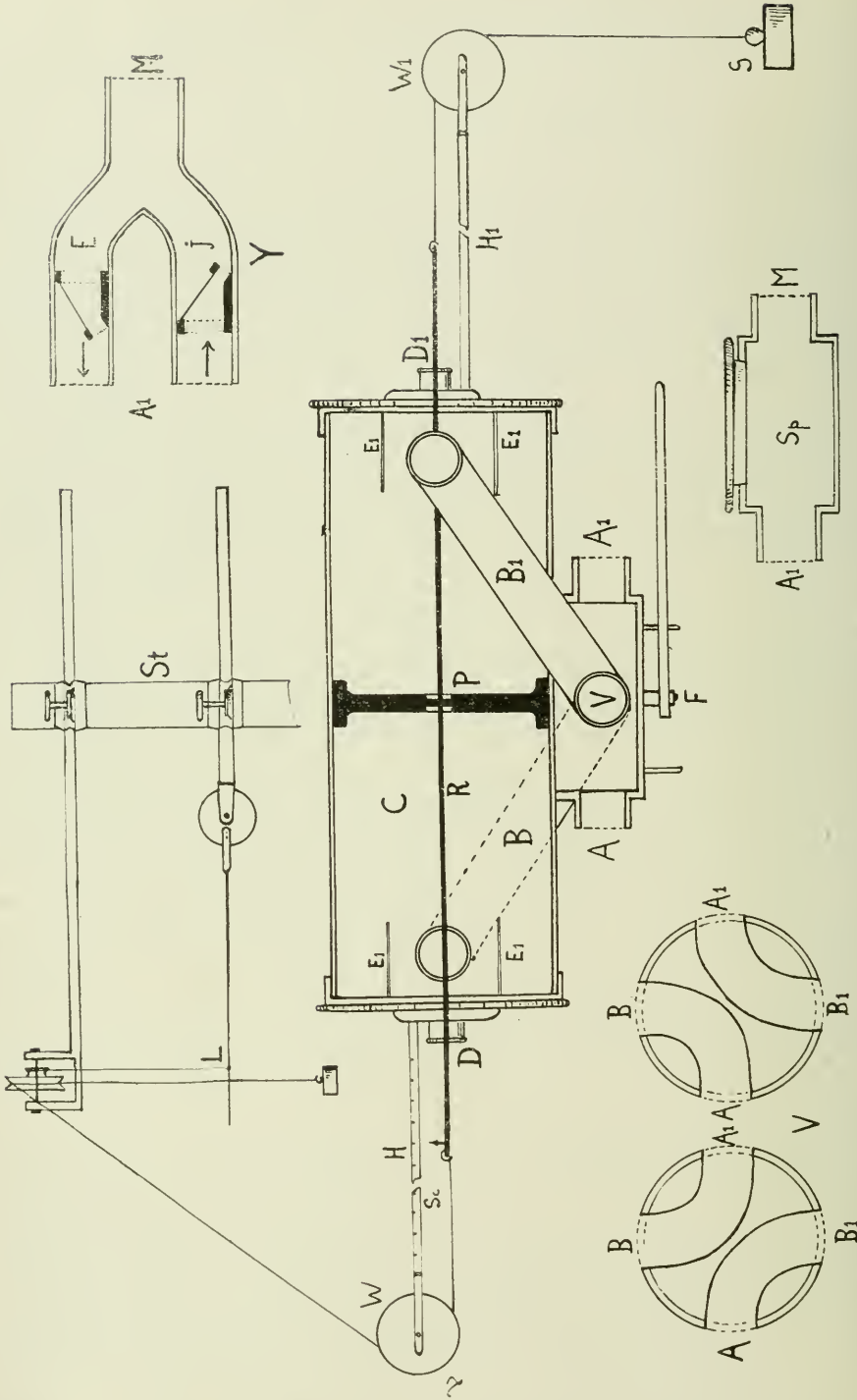


Fig. 1.—Recording spirometer; C, cylinder with piston and recording attachments; Y, inspiratory and expiratory valve; I', valve for changing the direction of the current of air; Sp, other chamber.

its center serves as a means of changing the position of these tubes, so that the air entering through orifice  $A_1$  may be diverted at any time either into tube  $B$  or tube  $B_1$ . Two small metal projections upon the floor of the capsule containing the disc indicate the positions which the handle of the axis must assume in order to cause the air to flow alternately in these directions.

If the entering air is diverted from tube  $A_1$  into tube  $B$ , the piston will be made to move from left to right. As soon as it has reached its extreme position against the thin rods  $E_1$  attached to cover  $D_1$ , the position of the rotating disc is quickly changed. Orifice  $A_1$  is thereby brought into communication with tube  $B_1$ . The air now rushes into the compartment below cover  $D_1$  and causes the piston to move from right to left. The rotating disc is again reversed as soon as the piston has attained its extreme left position. The air escapes from the cylinder through orifice  $A$ .

The reversal of the rotating disc consumes only about 0.25 second, and hence, if necessary a continuous stream of air may be made to traverse this instrument for almost any length of time. As has been stated above, the capacity of the cylinder is 2000 c.c. Accordingly, from three to four ordinary inspiratory or expiratory blasts of air may be directed through it before the disc need be rotated to allow another 2000 c.c. of air to enter. The same procedure is followed in measuring the complemental air and the vital capacity. Furthermore, it will be evident from the figure that the air may also be made to enter through orifice  $A$  and to escape through orifice  $A_1$ .

In order to be able to measure the inspiratory and expiratory amounts of air separately a Y-shaped tube has been constructed which may be attached to orifice  $A_1$  as well as to orifice  $A$ . The short limbs of this tube possess an internal diameter of 2.3 cm., so that their ends may be pushed over the inlet tube of the rotating disc. Each limb contains a valve, possessing in general the form of a slanting trap-door. Its narrow frame of brass fits snugly into the tube, while its orifice, measuring about 1.5 cm. in diameter, is closed by a round plate of aluminum, hinged above and slightly weighted below. Thus, the quick closure of this valve is insured not only by the slanting position of its metal disc, but also by the fact that the lower margin of the latter is equipped with a slight weight.

Naturally, these valves must be adjusted in such a manner that they open in opposite directions to one another. Thus, if it is desired to direct solely the inspiratory air into the cylinder to be registered, that limb of the Y-shaped piece of tubing must be connected with tube  $A_1$  which gives lodgment to the valve opening towards the mouth-piece  $M$  ( $J$ ). At this time, the limb ( $E$ ) containing the valve opening away from  $M$ , is permitted to project free into space. The expiratory air now escapes through this limb without being registered by the piston. Contrariwise, if it is our intention to ascertain the volume of the expiratory air, the tube containing valve  $E$  must be connected with tube  $A_1$  of the rotating disc, while tube  $J$  is permitted to project free into space. It need scarcely be mentioned that the position of this Y-tube may also be reversed, thereby bringing the valves  $J$  and  $E$  closer to the mouth of the subject. Their action is then reversed.

The free end of the Y-tube is connected by means of a rubber tube with a facial mask (*M*), such as is commonly employed in etherizing patients. An even more efficient appliance is the gas-mask, because it may be applied more firmly to the surface of the face. The internal diameter of this rubber tube should equal that of the metal tubing used in the construction of the spirometer. It need scarcely be mentioned that this spirometer may also be connected with the lateral of a tracheal cannula, enabling us to register the amounts of air breathed by an etherized animal. Lastly, it may be employed to record the amounts of air delivered by an artificial respiration machine. The trachea of the animal should then be connected with orifice  $A_1$  of the rotating disc, while the tube conveying the air from the pump should be attached to inlet *A*.

In order to be able to continue the narcosis for a long period of time, outlet  $A_1$  of the rotating disc may be equipped with a small metal chamber

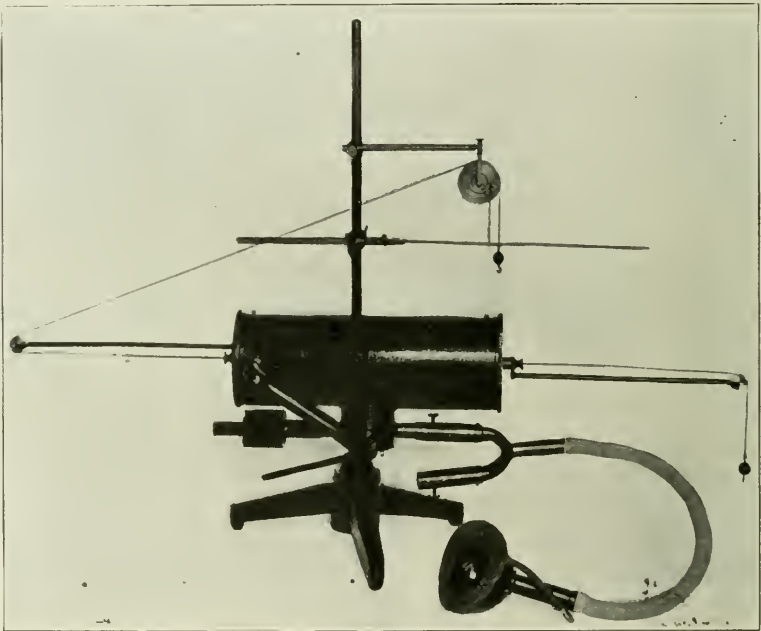


Fig. 2.

containing a sponge which may be moistened from time to time with ether (*Sp*). This chamber may be bulbular in shape, measuring about 5 cm. in diameter, or square with sides measuring about 5 cm. in length. Its cover should be made to fit very closely so as to prevent all leakage of air.

The volumes of the respired air are registered by means of a writing lever (*L*) which is connected with the end of the piston-rod (*R*) by means of a thread. This thread rests in the groove of wheel *W* attached to rod *H*. It is then wound twice around a second wheel placed directly above the tip of the writing lever. Its end is permitted to hang free in space weighted with a 3 gram weight. The second wheel measures 7.5 cm. in diameter, and is connected with a third wheel, rotating upon the same axis. The latter possesses a diameter of 2.5 cm. Its groove gives lodgment to a thread which is fastened to



the writing lever near its tip. This arrangement decreases the excursions of the writing lever considerably, so that the movements of the piston-rod which normally measures 22 cm. in length, yield a curve only 10 cm. in height. Accordingly, two tracings may be made above one another upon paper possessing the usual breadth of 25 cm.

The writing lever consists of a delicate beam of wood, measuring 28 cm. in length. Its weight is accurately balanced by the weight  $G$  attached to the other end of the piston rod. Inasmuch as rods  $H$  and  $H_1$  are movable, the recording lever  $L$  may also be connected with the right end of the piston-rod, while weight  $G$  is attached to its left end.

Another way of recording the volumes of the air respired is to attach a small weight to each end of the piston-rod and to equip one of these with a delicate writing lever which may be held against the paper of the kymograph by a horse-hair weighted at its lower end. Lastly, the volumes of the air may be read off directly from a scale ( $Sc$ ) attached to the guard-rod  $H$ .

Possibly the simplest way of calibrating the cylinder of this spirometer is to attach a large rubber tube to orifice  $A$  of the rotating disc and to permit its

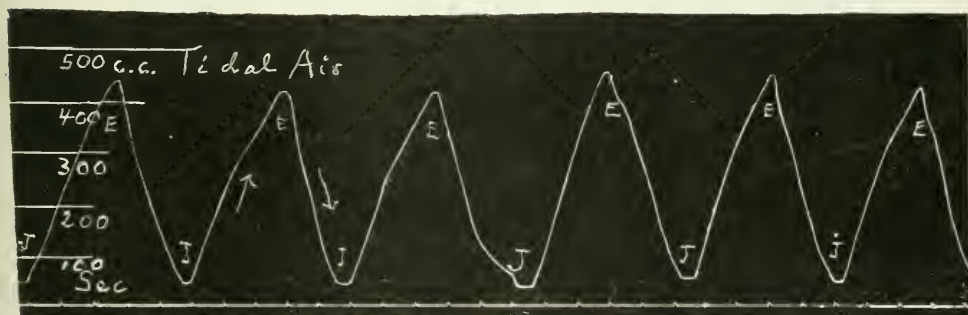


Fig. 3.

free end to project into a vertical graduated glass tube filled with water. If valve  $Y$  is now adjusted to yield only expiratory currents of air, the volume of each may be accurately ascertained by permitting this air to displace the water within the graduated tube.

If the facial mask  $M$  is connected directly with tube  $A$  or  $A_1$  of the rotating disc, the writing lever  $L$  will register a series of successive up and down strokes which may be widely separated from one another at their bases by increasing the speed of the drum. Since the course of the air determines the direction of the movement of the piston, the upstroke may represent either the inspiratory or the expiratory phase. In other words, the direction of movement of the writing lever depends upon the position of the rotating disc. To illustrate this statement more fully, I have inserted in this place Fig. 3, in which the letters  $J E$  indicate the inspiratory period, and the letters  $E J$ , the expiratory period. The time consumed by these movements is indicated by the pointer of a Jaquet chronograph. This record serves at the same time as the abscissa for the curve of the volume of the respired air. If the height of these



amounts in this instance to 1800 c.c., while the vital capacity (*CD*) equals 3200 c.c.

Under the same general conditions, the insertion of the expiratory valve yields a curve such as is represented in Fig. 5. The expiratory phases are here indicated by the letters *E J*, while the inspiratory phases (*J E*) are registered as straight lines, because these currents of air are not permitted to traverse the cylinder. Contrariwise, the interposition of the inspiratory valve causes the inspiratory blasts of air to be recorded but not the expiratory ones. The general character of the curves is the same in both instances.

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### A SELF-FEEDER FOR RATS\*

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BY RALPH HOAGLAND, B. AGR., WASHINGTON, D. C.

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ONE of the most important pieces of equipment needed in carrying on quantitative feeding tests with rats is a self-feeding device that will allow the rat free access to the feed and yet prevent his wasting it or contaminating it with his excrements. The writer has devised a feeder which meets the above requirements satisfactorily and a description of the same is offered for the benefit of any one interested in such a piece of equipment.

Fig. 1 is a scale drawing of the feeder. It consists of three separate parts designated as: *A*, receptacle for waste feed, also serving as base for feeder; *B, C.*, vestibule and feed hopper; *D*, cover to feed hopper. The feeder is made of heavy-coated bright tin and one-eighth inch mesh galvanized-wire screen. The respective parts of all feeders are interchangeable. A good tinsmith can make one of the feeders in approximately two hours.

The feed hopper will hold approximately 100 grams of feed, depending upon its character. When the ration is relatively low in fat the feed will drop down as the rats eat, but when the fat content is high and the feed rather sticky, it is necessary to knock down the feed once daily. At present the writer is adding ten per cent of fat to the rations and the feeders are examined daily to see that ample feed is available. The feed hopper is filled with feed and then weighed. At the end of a week the feeder is freed from any adhering feces and weighed. The loss in weight represents feed consumed, provided there has been no wastage. The screen floor to the vestibule catches the feces so that they do not contaminate any feed that may have fallen into the base of the feeder.

The vestibule to the feeder is larger than necessary for a young rat weighing 40 to 50 grams, but it will accommodate a mature animal weighing 250 to 300 grams. However, the feeder has given satisfaction with small rats as well as with large ones, so that only one size has been used. In fact, the

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\*From the Biochemic Division, Bureau of Animal Industry, United States Department of Agriculture, Washington.

Received for publication, December 1, 1921.

large rats have been the worst offenders in pulling down feed into the base of the feeder.

For the most part, the rats do not pull out any considerable quantity of feed into the waste-feed receptacle, particularly if the ration is adequate, and

## SELF-FEEDER FOR RATS

Made of Tin Plate and  $\frac{1}{8}$ " mesh wire screen.

A: Receptacle for catching waste feed.

B: Wire-screen Vestibule to Hopper.

C: Feed Hopper.

D: Cover for Hopper.

a-b: Opening giving access to feed.

b-c: Baffle.

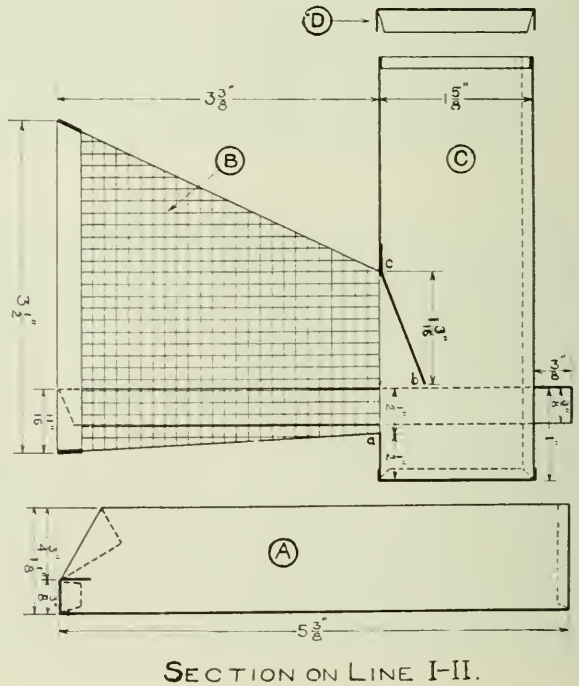
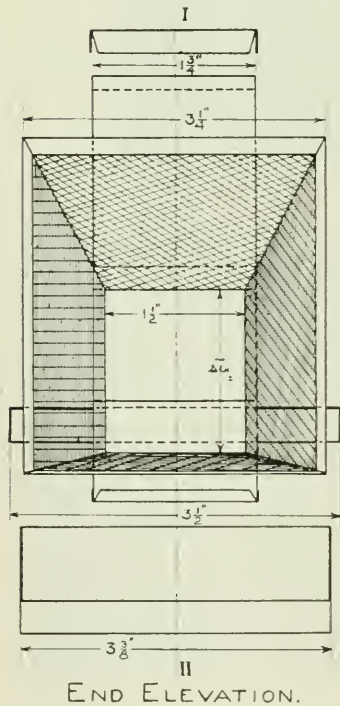
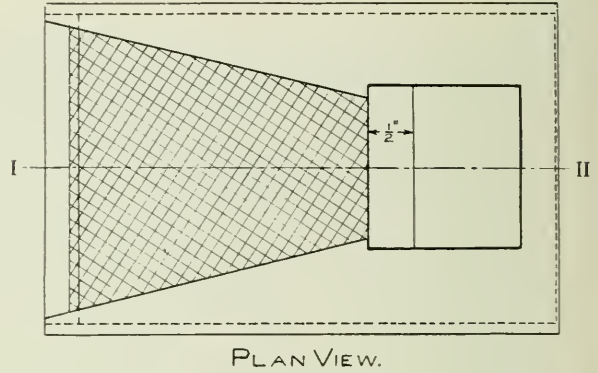


Fig. 1.

it is only occasionally that a rat will pull a little feed entirely out of the feeder on to the floor of the cage. In our feeding tests, each feeder is examined daily, feces being removed from the floor of the vestibule and any feed in the waste-feed receptacle, unless unclean, is returned to the hopper, which is tapped to knock down the feed within reach of the rat. The feeder is weighed at the



end of the week and the feed remaining in the hopper is transferred to a clean feeder, which is filled with additional feed and weighed. The dirty feeder is cleaned and then sterilized in live steam. A reserve supply of approximately one-half the number of feeders in use is maintained so as to facilitate operations.

In the light of several months' experience with the self-feeder described (approximately eighty are now in use), the following slight modifications are suggested: (1) Increase the depth of the waste-feed receptacle (A) from  $1\frac{1}{8}$  to  $1\frac{1}{2}$  inches, so as to provide more storage room for waste feed; (2) modify the baffle plate (b-c) so that it can be raised or lowered as desired in order to prevent rats from pulling feed out into the vestibule.

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## REMOVABLE WATER-BATH TOPS FOR PARAFFIN EMBEDDING AND LOW-TEMPERATURE EVAPORATION\*

BY OSCAR T. SCHULTZ, M.D., CHICAGO, ILL.

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THE writer described in 1917† a rectangular electric water-bath for serologic work. There are now on the market a number of baths constructed after the general plan there described. The need having arisen in our own work for a bath for paraffin embedding, it appeared possible to devise a top which would transform the serologic water-bath into a paraffin bath at a very considerable saving of space as compared with the usual incubator or cabinet form of paraffin embedding oven. With this object in view we have had made according to our specifications a removable top to fit the small 9 by  $4\frac{1}{2}$  by 5 inch water-bath. The top (Fig. 1) is made of sheet copper and has three cylindrical, sheet copper depressions,  $2\frac{3}{4}$  inches deep by  $2\frac{1}{4}$  inches in diameter, each of which holds a beaker of stock size. There are also five depressions  $1\frac{3}{4}$  inch deep by  $1\frac{1}{16}$  inch in diameter which take wide-mouth vials or square bottom test-tubes of standard sizes. A tubulature is provided for a thermometer. Thus arranged, a bath (Fig. 2) which requires an extreme table space of only 12 by 6 inches has been found ample for the pathologic work of a 475-bed hospital in which all tissues removed at operation are routinely sectioned.

It may not be amiss to describe the method which makes possible the running through of such an amount of tissue with the minimum of trouble and labor and with no danger of confusing specimens. Every specimen, as soon as it is received from the operating room, is given a surgical pathology number, the data relating to the specimen are entered upon the proper record forms, the gross material is examined fresh, and then it is placed in the fixing fluid. After fixation and after the blocks of tissue for microscopic examination have been trimmed and are ready for dehydration, a small paper label bearing in pencil the number of the specimen is attached by means of a small drop of melted

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\*From the Nelson Morris Memorial Institute for Medical Research of the Michael Reese Hospital. Received for publication, Dec. 20, 1921.

†Schultz, O. T.: An Electrically heated, Constant Temperature, Water-Bath for Serologic Work, *Jour. Am. Med. Assn.*, 1917, Nov. 1, lxxix, 1521.

gelatin to the flat side of the tissue block opposite to the side from which the sections are to be cut. After allowing the gelatin to harden for a moment or two, the tissues are placed in the first dehydrating alcohol. In this way all the blocks from the material of a single day are handled together and are run through the various dehydrating and clearing fluids and into the embedding bath. A slip of paper which bears the specimen numbers of the tissues included in a given lot and the number of blocks from each specimen is carried along with the tissues. After embedding in paraffin, the specimen number can be read through the layer of paraffin which covers the small label on the reverse side of the specimen: this number is written in pencil or ink on the fiber block on which the tissues are mounted for sectioning. The individual blocks of

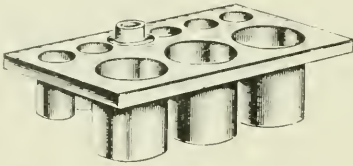


Fig. 1. Removable top for paraffin embedding.

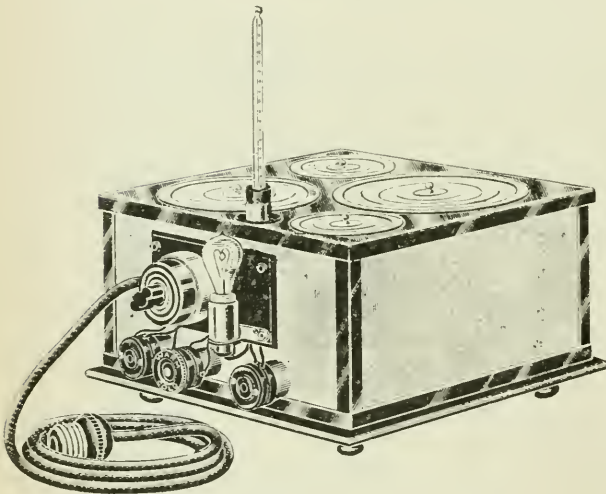


Fig. 3.—Water-bath with ring top.

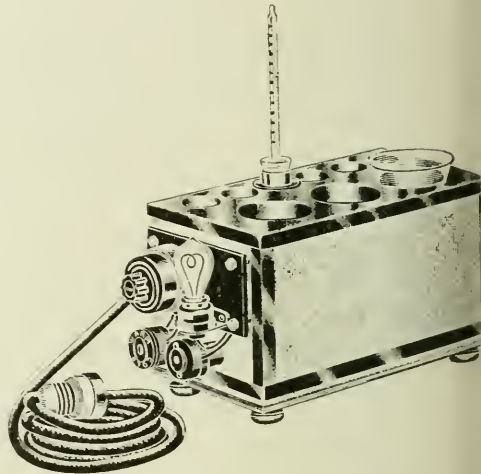


Fig. 2.—Water-bath complete for paraffin embedding.

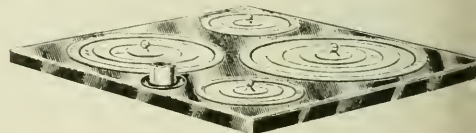


Fig. 4. Removable ring top.

tissue from autopsies are not numbered, the entire lot from a single autopsy going through the various procedures together. Tissues which are to be examined for special investigative purposes are numbered like the surgical material and can be carried along with the latter. Very minute specimens do not have a label attached; they are handled separately and are embedded in the smaller receptacles which the paraffin bath provides.

In addition to the paraffin bath top, we have had made a removable ring top which transforms the 9 by 9 by 5 inch serologic bath into a bath for evaporation of volatile fluids at low temperature (Fig. 3). The top (Fig. 4) is made of sheet copper and has two openings 2 and 1 2 inches in diameter, one opening 4 inches in diameter and one opening 5 inches in diameter. Each opening is fitted with a series of concentric rings by means of which the size of the opening may be adjusted as required.

## AN INJECTION METHOD FOR AIDING IN THE IDENTIFICATION OF TAPEWORM SPECIES\*

BY RAPHAEL ISAACS, M.D., CINCINNATI, OHIO

A SIMPLE method for visualizing the uterus in tapeworm proglottids, to identify the species, or for demonstration purposes, as used in the wards and laboratories of the Cincinnati General Hospital for the past five years, consists in the injection of the canals with India ink. A hypodermic syringe (1-2 c.c.), fitted with a fine needle, is filled with India ink. The segment to be

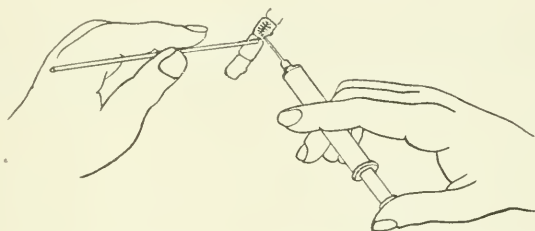


Fig. 1.—Method of holding and injecting tapeworm segments.



Fig. 2.

injected is held flat on a piece of glass by means of a wooden match stick or applicator, and the needle inserted into the substance of the fresh proglottid, near the lateral genital pore. With a little manipulation, one of the diverticulae of the uterus is easily entered, and the ink readily fills all the branches. Pressure between two glass slides brings out the details very clearly, and the

\*From the Department of Internal Medicine, Cincinnati General Hospital, College of Medicine, University of Cincinnati.

Received for publication, December 17, 1921.

sparingly branched organ of *T. solium* is easily differentiated from the more abundant ramifications of *T. saginata*. The method is also applicable to other cestodes, and may also be used with preserved material although the results are not as complete as with fresh. Permanent preparations may be made by preserving the flattened segment in 10 per cent formaldehyde, dehydrating in alcohol, and, after clearing in xylol or carbol-xylol, mounting in balsam.

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## A METHOD FOR THE SEPARATION OF SPORE-BEARING ANAEROBES FROM OTHER SPORE-BEARING BACTERIA\*

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BY J. HOWARD BROWN, PH.D., PRINCETON, N. J.

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IN the isolation of anaerobic spore-bearing bacteria the strictly aerobic bacteria are easily disposed of because they fail to grow under strictly anaerobic conditions. The heating of mixed cultures or of a suspension of milk or feces for twenty minutes at 80° C. in sealed tubes is the common method of separating sporulating from nonsporulating organisms. If carried out carefully this method serves to kill the vegetative bacteria leaving only the spores to germinate when transplanted to suitable media. In our experience the separation of the anaerobes from the facultatively anaerobic spore-bearing bacteria was attended with considerable difficulty until the method to be described was employed. Many of the facultative anaerobes grow well under anaerobic conditions. Many of them are surface spreaders on the blood agar plate. The fact that under anaerobic conditions they may not present the same typical appearance that they do under aerobic conditions makes their recognition so much the more difficult. There may be nothing about their colonies to distinguish them from anaerobes growing in the same plate. We have frequently fished numerous colonies from an anaerobic blood agar plate and found them all to be facultatively anaerobic spore-bearing bacteria.

Various means of overcoming these difficulties have been tried. Preliminary incubation of mixtures under aerobic conditions to effect the germination of the spores of aerobes, followed by exposure to heat to kill the vegetative forms was attended by some success. However, much better results were obtained by incubation of the material in fluid media under strictly anaerobic conditions for several days, then heating the mixed culture for twenty minutes at 80° C. It was found that under strictly anaerobic conditions the facultative anaerobes did not produce spores, or at least none were found within the period of incubation of one week. Incubation must be long enough to permit the anaerobes to produce their spores. Florence (1922) has found that even under partially anaerobic conditions spore formation by a variety of aerobes and facultative anaerobes is greatly delayed. She says, furthermore, that "if transfers were made from cultures containing spores, the majority of spores carried

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\*From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.

Accepted for publication, February 9, 1922.



over did not germinate in the sealed tubes." We have not studied this phase of the problem, nor do we wish to state that no spores are formed by facultative anaerobes under anerobic conditions of growth. However, if certain of such spores do survive the process of anaerobic cultivation and the subsequent heating they are not present in sufficient numbers to cause serious trouble in the isolation of the anaerobes. After the vegetative forms have been killed by heat the mixture may be plated anaerobically at once or after germination of the spores in fluid media. For the preliminary anaerobic culture various selective media may be used to enhance the growth of various groups of anaerobic bacteria. For obtaining the growth of a large number of anaerobes of various kinds we have found it advisable to inoculate original material into at least the following three media under vaseline in test tubes, (1) cooked meat medium, (2) dextrose bouillon, and (3) sugar-free bouillon. Sterile tissue may be added to the last two if desired.

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# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, AUGUST, 1922

No. 11

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## EDITORIALS

### *The Diabetic Diet*

THE principle of undernutrition in the treatment of diabetes, first elaborated by Allen and by Joslin, has, since 1914, been the most successful guide to intelligent therapy. Its advantages over previous methods have been demonstrated beyond cavil. By its use drugs and patent medicines have been largely eliminated and diabetic coma has been reduced to a minimum. Alkali treatment has passed largely into disuse. The average life of the diabetic subject has been increased by about two years. Joslin points out that with one million diabetic patients in the United States this treatment has added two million years in the aggregate to their lives. The hospital mortality rate has dropped from 28 per cent before 1914 to 2 per cent in 1920. This is due in large part to the prevention of coma but also to extraneous factors such as the more frequent hospitalization of early cases, for clinical observation. A diabetic under proper

treatment may now look forward in fair security to from five to seven years of life. Cases recognized early, such as those diagnosed during life insurance examinations have averaged twelve years of life. Under Joslin's care children now live fully three times as long with diabetes as they did prior to 1914, and the duration of the disease in the young adult is extended at least one-half. According to Allen an individual developing diabetes after forty or fifty may now look forward to completing his normal expectancy of life. The increased duration is not only common to all decades but is greatest in the decade with the most severe cases, namely the first.

Between 1914 and 1921 the Joslin and Allen treatments were used almost exclusively in this country. Since then considerable attention has been given to possible improvements in these diets. Joslin himself emphasizes the danger inherent in too strenuous fasting of the diabetic subject. In a recent article he states that although it may be desirable to lower the weight slightly, extreme lowering can hardly be advantageous. The ravages of a day's fast are not quickly replaced. A man on complete fasting loses 0.7 per cent of his body tissues each day. In a diabetic this cannot be quickly made up by overfeeding. One cannot give more than 5 or at most 10 per cent of extra food. Thus it may require as long as twenty days to make up for a single day of starvation, and the loss of weight entailed by a five days' fast might require from a hundred days to even a year for complete recovery. Weight must be sacrificed in order to get the diabetic sugar-free, but beyond this there should be no unnecessary loss. For each kilogram of body weight lost the patient loses approximately 126 grams of protein, 269 of fat and 15 of carbohydrate.

Thus the cost of undernutrition is great. Joslin recognizes its disadvantages but insists that even with these the ultimate results are greatly superior to those following overfeeding. He has modified his own preliminary treatment by omitting actual fasting whenever possible, and substituting partial fasting combined with a fat free diet. Frequently this suffices to free the urine of sugar. The danger from acidosis is diminished and patients leave the hospital sooner than after complete fasting.

Newburgh and Marsh in December, 1920, first published their observations on the treatment of diabetes with diets high in fat as compared with the Allen and Joslin diets. An apparently radical departure from the older methods and antithetical in principle, this method has on the contrary many points of resemblance. In both systems the principle of undernutrition is maintained. In the Allen or Joslin diets the carbohydrate intake is as high as is consistent with the individual's tolerance and the fat intake is relatively low. The reverse holds true in the dietary proposed by Newburgh and Marsh. The latter also adjust the nitrogen intake to a point not higher than is requisite to maintain nitrogenous equilibrium. A protein intake above the minimum is undesirable because of the glycogenic capacity and the specific dynamic action of the protein. Diabetics do not require more protein for the establishment of a nitrogen balance than do normal subjects. This, however, is not a point of distinct difference from the Joslin diet. Their modifications consist briefly in a reduction of the protein ration by about twenty grams, and the use of a fat and total

calory allowance somewhat higher than that of Allen or Joslin, but distinctly lower than that of Naunyn, Von Noorden and Petrén. They believe that by these modifications they can establish a higher tolerance or at least furnish a higher caloric intake, thereby preserving more satisfactorily the patient's nutrition.

Woodyatt has elaborated on the work of Newburgh and Marsh and has stressed the necessity of treating diabetes in terms of metabolism rather than of diet. It is true that protein, fat and carbohydrate are the substances ingested, but it is not these substances, as such, that find their way into the body tissues. Starch in the intestines and glycogen in the liver both appear as glucose in the blood. The protein of the diet no longer exists as such after digestion and absorption. Even the resultant amino acids are ultimately resolved into various components, of which glucose and beta-hydroxybutyric and acetoacetic acids form a large part. From 100 grams of carbohydrate the body, theoretically, metabolizes 100 grams of glucose. From 100 grams of protein the body metabolizes 58 grams of glucose and according to Woodyatt around 46 grams of fatty acids. One hundred grams of fat yield in the body approximately 10 grams of glucose coming from the glycerol and 90 grams of fatty acids. All of the foods of the diet except a small fraction of the proteins resolve themselves in the body into two substances, glucose and the higher fatty acids.

Carbohydrate tolerance therefore cannot be estimated simply from the carbohydrate of the diet. Estimations must also include the glucose derived from protein and fat, and the glucose arising in the body from endogenous sources.

Another fact on which Woodyatt lays great stress is the importance of the ketogenic balance in the body. Shaffer believed that acetoacetic acid itself is not easily burned in the body but that in combination with glucose or some substance intimately related to glucose an easily oxidizable compound is formed. Those substances which during metabolism give rise to acetoacetic acid and related acid bodies have been termed ketogenic. Those furnishing glucose which will combine with these acetone bodies are termed antiketogenic. Ketogenic substances are derived from fat and from certain amino acids such as leucin, tyrosin and phenylalanin. Antiketogenic substances are obtained, as we have seen, from glucose, protein and the glycerol fraction of the fat.

A balance must be maintained between the ketogenic and the antiketogenic substances. A preponderance of the former will cause acidosis. Thus if the glucose tolerance in an individual falls so low that one cannot administer sufficient glucose or antiketogenic substance to neutralize the ketogenic, acidosis will result. To prevent this the ketogenic intake must be decreased.

According to this conception we must estimate both sugar and fat tolerance in diabetics. The appearance of abnormal quantities of acetone, acetoacetic acid and beta-hydroxybutyric acid is the immediate result of the oxidation of certain fatty acids in the absence of a sufficient proportion of oxidizing glucose. For each individual there exists a definite ratio between the quantity of glucose oxidizing in the body and the maximum quantity of ketogenic fatty



acids that can be oxidized in the same time without the appearance of abnormal amounts of acetone bodies. Shaffer suggests that one molecule of glucose is necessary for the complete oxidation of one molecule of acetoacetic acid or one molecule of any higher fatty acid or amino acid capable of yielding one molecule of acetoacetic acid. Woodyatt assumes for clinical purposes that the ratio of higher fatty acids to glucose which, if exceeded, will lead to acidosis is about 1.5 to 1 (in grams). Wilder places this ketogenic threshold ratio at 2 to 1.

The rationale of dietetic management is then, according to Woodyatt, the adjustment of the glucose intake to within the carbohydrate tolerance and the adjustment of the fatty acid supply in relationship to the glucose supply so that the ketogenic balance is such that the ratio of 1.5 to 1 shall not be exceeded. In this way the patient not only becomes sugar-free but acidosis is insured against. The fat intake may be high provided it is not so high as to furnish an excess of fatty acids above that allowed in the ketogenic ratio.

Lusk stated in 1917 that in a fasting individual with much fat, little protein was consumed, but that when there was little fat much protein was burned and when there was no fat, protein alone yielded the energy of life. Woodyatt points out that the ingestion of fat may spare tissue fat and thus prevent the protein loss from becoming abnormally great. Fat ingestion may diminish the protein loss in those cases where the tissue fat has become too much depleted. If a fasting patient mobilizes and burns from 100 to 120 grams of fat the administration of an equal amount through the diet serves merely to conserve the tissue fat by that much. Why should the patient be compelled to draw from his tissues the fat that he might draw from a diet, particularly when in doing so he may increase his protein losses?

Woodyatt, in short, has attempted to arrange a diet in which the protein is adjusted to maintain nitrogenous equilibrium, the ketone products are completely oxidized, the available carbohydrate does not exceed the glucose tolerance, and which contains the greatest possible number of calories for the individual. In the compilation of such diets rather complicated mathematical formulae are necessary. This part of the work has been simplified by graphic charts which have been prepared by O'Hara, by Hannon and McCann, and by Wilder. With any of these the determination of the patient's optimal diet becomes comparatively easy.

Wilder emphasizes the importance of maintaining a decreased metabolic rate in cases under treatment. With a low sugar tolerance the diet must be carefully balanced so that the fatty acid-glucose ratio will not be so great as to cause acidosis. Reduction in the total metabolism will decrease the production of fatty acid molecules. One great advantage of caloric restriction probably lies in the consequent reduction of metabolic rate. It has been quite definitely shown that sugar tolerance in the diabetic may be increased by measures which depress the level of basal metabolism. Starvation causes a sustained fall in metabolism up to a point when, with extreme starvation, the rate tends to rise. Wilder suggests that when the fat stores are exhausted, the body proteins are called upon to provide the energy and the specific dynamic action of this cata-

bolized protein serves to elevate the metabolism. When this stage arrives in the severe diabetic, the effect on sugar tolerance is disastrous.

Fortunately a decrease in the metabolic rate may be obtained by undernutrition as well as by the actual fast. Wilder states that a low protein diet providing calories 20 per cent below the calculated caloric requirement will accomplish a depression of basal metabolic level of from 15 to 25 per cent. Thus the depression of the metabolism equals and balances the caloric deficit of the food, and both theoretically and practically patients may be maintained at constant weights on such diets. In maintaining a low metabolic rate the protein intake should only be sufficient to maintain nitrogen equilibrium so that its specific dynamic action will be minimized. Wilder accepts Newburgh and Marsh's conclusions that for an adult two-thirds of a gram of protein per kilogram body weight is sufficient.

In discussing the ketogenic-antiketogenic balance he points out that unless the calory and nitrogen values of the diet approximate the calory and nitrogen requirements of the patient, the results will show an unexpected imbalance in the ratio. Thus a diet may be accurately calculated and may contain the correct ketogenic-antiketogenic proportions, but if its caloric value is decidedly below the minimum requirement of the patient the deficiency will be made good at the expense of endogenous food stores, chiefly fat.

The dietary modifications above enumerated have not been unqualifiedly accepted by Joslin or by Allen, both of whom have had a volume of experience which adds value to their criticisms. Joslin remarks that just as experience with undernutrition has steadily yielded better results in his hands so, under certain conditions has the more liberal use of fats. He enumerates as the minimal requirements for the acceptance of any new method of treatment, first that the duration of the disease shall exceed the present average of 5.3 years, second that by the new treatment no deaths will occur during the first year of the disease, and third that the mortality from coma among patients treated in hospitals shall not exceed 1 per cent.

He remarks that a high fat, low carbohydrate diet is not new but represents the Naumyn type of treatment. The difference between the two lies in the relatively low quantity of protein and the reduction of total calories in the newer method. All past evidence indicates that the low carbohydrate-high fat ratio was responsible for the former high mortality from acidosis. He questions whether the reduction in protein and calories will prevent this mortality in the future. That coma was prevented in the series treated by Newburgh and Marsh is attributable to the low caloric value of the diet. Joslin points out that the dietaries used by Newburgh and Marsh contain a proportion of fat to carbohydrate twice that considered safe by Woodyatt.

Joslin particularly takes exception to the abnormal ratio between carbohydrate and fat. This ratio in the normal diet is about 6 to 1 while in the Newburgh and Marsh diet the proportion is almost reversed. A diet so low in carbohydrate and so high in fat palls upon a diabetic after several years of use. He points out that it has not been conclusively proven whether a patient acquires increased carbohydrate tolerance more readily by a high carbohydrate-

low fat ratio or by the reverse. "I cannot escape from the impression that a diabetic patient whose carbohydrate is once radically lowered and fat is radically raised suffers from the process and seldom regains a high carbohydrate tolerance."

Allen and Sherrill remark that the only actual modification suggested by Newburgh and Marsh is a reduction of the protein ration by perhaps twenty grams, and the use of a fat and total calory allowance somewhat higher than that of Allen and Joslin but lower than that of Naunyn, Von Noorden and Petré. They do not accept the statement that since fat is burned in the fasting body the feeding of sufficient fat merely preserves the body fats. "Such suppositions defy old and new knowledge of both metabolism and diabetes." On a low carbohydrate-high fat diet the liver and the body tissues in general become stored with fat in preponderance over carbohydrate. The general overload of metabolism gradually lowers the carbohydrate tolerance. Glycosuria ensues and wastes some of the scanty store of carbohydrate. Then, the excess of fat in catabolism easily precipitates coma. However, the diabetic free from glycosuria can endure larger quantities of fat than those used by Newburgh and Marsh with no immediately serious acidosis. It is the later effects that must be guarded against.

Allen and Sherrill evidently consider loss of weight as an important feature of undernutrition therapy. This appears to be at some variance with the conception of Joslin. "To save body fat is contrary to the principle of undernutrition treatment, which is that the body weight must be reduced in proportion to the severity of the diabetes, as the only effectual means of keeping diabetic symptoms under control." According to their conception apparently, an increase in the caloric value of the food is not particularly to be desired even though this can be accomplished without the production of glycosuria.

They consider the cases described by Newburgh and Marsh to be in the main mild and therefore of the type that would not show early unsatisfactory results. They emphasize that the Ann Arbor workers by no means allow unlimited quantities of fat and that they follow closely the principle of undernutrition. It is only in cases in which this principle has been neglected that unsatisfactory results have been experienced. "They have at least not fattened a single severely diabetic patient, restored him to any lasting usefulness, or even kept him alive any long time on any high caloric ration. The one essential evil from which the undernutrition treatment gave relief was excessive fat feeding, and the benefits of such relief appear more strongly demonstrated today than they were at the time when this treatment was first proposed."

At the present time we have in this country two schools of diabetic therapy, both using the principle of undernutrition, but differing chiefly with regard to the value of the fat-carbohydrate ratio. Apparently logical theoretical considerations exist in favor of either method. The newer method will probably become popular if for no other reason than because of its apparent simplification with the charts and tables already published. Joslin has by a similar procedure made his treatment equally easy of execution. The minimum requirements enumerated by Joslin for the acceptance of the new method as an im-

provement must be met. Unsatisfactory results will, according to its critics, be rather late in appearing and time will therefore be necessary before convincing conclusions can be produced. In the meanwhile those who use the relatively high diet must do so with an understanding of the metabolic processes affected and of the potential dangers inherent in its faulty administration.

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—W. T. V.

### A New "Tuberculosis Cure"

"GENEVA, July 22.—The British Red Cross, after careful investigation, has decided to purchase the vaccine treatment against tuberculosis originated by Henry Spahlinger, the Geneva biologist, and a contract for the sale has just been signed in London. The Spahlinger laboratories here will be continued, and others will be established, it is stated. A number of American patients, many of them advanced cases, and some given up by physicians, are now under treatment here, and all are showing improvement. "The Spahlinger discovery was announced in Paris early in 1914. Little more was heard of it until after the war, but within the last year or two, it has attracted considerable attention, notably on the part of British officials. The serum can now be made as cheaply as the ordinary smallpox vaccine."

(Associated Press Item in Richmond Times Dispatch, July 23, 1922.)

Search through the pages of the Quarterly Cumulative Index of Current Medical Literature, since January 1, 1920, reveals but one reference to this method of treatment. This consists of an article by Henry Spahlinger of the Bacterio-Therapeutic Institute, Geneva, appearing in the Lancet, London, January 7, 1922.

Spahlinger's treatment utilizes either or both of two therapeutic principles depending on the nature of the infection. In acute or rapidly progressing cases passive immunization with special immune sera is employed. Active immunization with various special antigens is practiced in chronic afebrile cases, in non-pulmonary forms and in cases "predisposed to" tuberculosis.

Passive immunization is described as a form of progressive disintoxication. Different symptoms of intoxication are supposed to be due, not to varying manifestations of the same toxin, but to the action of different distinct toxins against each of which an antitoxin or "partial serum" must be prepared. These toxins are not merely the normal products of metabolism, but also substances elaborated by the microorganism as a means of defense. They are obtained experimentally by the use of virulent strains of bacteria "stimulated at definite intervals by physicochemical means, such as variation of temperature, in order



to harry the existence of the bacilli and force them to produce some of their poisons."

The three types of "partial sera" prepared from these toxins are, (1) antitoxins "obtained by means of" ectotoxins, (2) antiendotoxins "obtained by means of poisons derived indirectly from the bacillary bodies," and (3) antibacterial sera produced by the inoculation into a horse of dead and, later, living microbes, after a preliminary prolonged and intensive vaccination. The proportions of these sera are varied according to the individual case. Thus, for pulmonary tuberculosis there must be a predominance of antitoxins, while for surgical tuberculosis antiendotoxins must predominate. In pulmonary tuberculosis antitoxins and antibacterial sera against the common secondary invaders are administered. In abdominal tuberculosis sera are also given by mouth. Injections are made directly into infected glands.

The aim of this antitoxic treatment is to destroy the poisons generated within the body, thereby enabling the body to more successfully elaborate its own defenses. In this way, the rapid development of the disease is stopped, and the condition is changed from an acute to a chronic one. It is not claimed that treatment by passive immunization is curative. "Although a clinical recovery is brought about by the serum, relapse or a re-infection is always possible because the passive immunization has not modified the natural resistance of the patient to tuberculous infection."

Active immunization is employed in the chronic forms of tuberculosis. "Various antigenic elements of the bacillary protoplasm are injected separately into the patient in order to secure a series of immunities against all the deleterious products of bacterial disintegration." The author emphasizes the importance of maintaining the strength and specificity of these partial antigens during their preparations. He states that in the preparation of the vaccine the ectotoxins must be removed from the bacillary bodies. He utilizes a mixture of antigens and antibodies in the belief that active immunization is thereby facilitated and that undesired reactions are avoided.

Various vaccine combinations are employed, but the final dosage must contain all the antigens. The treatment extends over a period of a few months.

The illustrative cases reported are eight in number and are characterized by some vagueness in description. This may be because the article is in the nature of a general summary. Nevertheless it is regrettable. Three roentgen plates are shown but they are on three different patients, so that no comparison can be made. The most striking improvement is found in case 4 which is reported as follows.

"October, 1916: Pulmonary tuberculosis diagnosed, went into the country. 1918: To Sabourins sanatorium, T. B. in sputum, temp. 100.4°-100.8° F. Returned to Paris not cured. Lost 11 lbs. in one month. To Riviera, gradually getting worse. April, 1919: In bed with temperature 102.4°; Haemoptysis lasting for three weeks. Up for a short period, then returned to bed where she remained until April, 1920, when condition was very critical. Violent and continuous cough, paroxysms lasting for as long as 20 minutes, expectoration green and purulent (350 c.c. per day). Drenching sweats, extreme emaciation, com-

plete anorexia. Pulse rate 140, temperature 105.8°, insufferable headaches, delirium; death imminent. April, 1920: intensive Spahlinger treatment. Four injections of partial antitoxin No. 2 were given per day (morning, noon, afternoon and evening) and 10 c.c. of serum by mouth 12-hourly. In 15 days the cough had practically ceased. Temp. fell from 105.8° to 99.5°, and the pulse-rate from 140 to 78. Amount of expectoration dropped from 350 c.c. to three or four daily expectorations. Appetite quickly restored. Night sweats diminished in frequency, but did not entirely cease until expiration of two months. Although after a fortnight nearly all signs of intoxication had gone, the intensive treatment was continued for a further period of one month and then gradually reduced to two injections of serum weekly. In August the patient came to Geneva. She gained 1st 10 lbs. in four months. X-rays showed total opacity of the whole of the left lung and the right base. September, 1920: Examination showed no pulmonary activity. April, 1921: Re-examined, no active physical signs, patient cured."

—W. T. V.

### *The Etiology of Rickets*

POSSIBLY our readers may be inclined to the opinion that there is at present in current medical literature an excess of articles on this subject, but, after reading a very interesting review by Findlay\* we cannot resist the temptation to briefly run over the theories concerning this disease. These may be grouped as follows:

(1) *The Calcium Deficiency Theory.*—In the first study of rickets it was quite evident that the most characteristic and striking pathologic condition is the deficiency of calcium in the bones. This might result from any one of the following conditions: (a) a deficient supply of calcium in the food; (b) imperfect absorption of calcium from the intestinal canal; (c) inability on the part of the growing bones to fix calcium in their substance, and (d) inability on the part of the bones to retain the calcium assimilated. Following this theory, rickets was believed by many to be due to deficiency of calcium in drinking water. Many investigators attempted to induce this disease in animals by reducing the amount of calcium in the food. Since calcium is an essential constituent of the bones, if there be none or an inadequate amount in the food, the bones cannot grow normally. This follows as an incontrovertible fact. The same thing is true of phosphorus. Some of the early experimenters, notably Roloff and Voit, on calcium deficient diets induced in animals changes which they believed to be those characteristic of true rickets, but when the etiology of the disease was better understood the results following deficient calcium diets were shown to be merely an osteoporosis. Moreover, experimentation soon demonstrated that rickets does occur sometimes in animals in which there is no calcium deficiency in the food and no imperfection in absorption can be demonstrated. It is evident that there is still much work to be done under the

\*The Lancet, 1922, i, 825.

calcium deficiency theory. Most of the experiments on this theory have been carried on for too short a time to justify final conclusion. It is easy to demonstrate, as has been done repeatedly, that rickets may develop when the food contains even an excess of calcium, but this throws no light upon the question of either the assimilation of the calcium by the bone or the bone's ability to retain the calcium which has been absorbed. Wells has shown by introducing cartilage into the peritoneal cavity of a rabbit that the calcium is absorbed in proportion as it is destined to become calcified. Findlay and his colleagues have repeated this experiment and have found that rachitic cartilage absorbs more calcium than does normal cartilage. Kramer and Howland find no alteration in the calcium content in the blood in rickets, but have been able to detect a very definite deficiency of inorganic phosphorus.

(2) *The Infection Theory*.—Many years ago the claim was made, notably by certain French and Italian observers, that rickets is an infectious disease, and in 1911 Robert Koch stated that he could induce rickets experimentally in animals by the injection of streptococci. More exact study of this matter has shown that the changes induced by a chronic poisoning with a streptococcus protein have not the slightest resemblance to rickets. Findlay and colleagues at the University of Glasgow have attempted to obtain evidence of a specific antibody in rickets by means of the complement deviation test. They employed as antigen, watery and alcoholic extracts of rachitic cartilage from the ends of long bones and ribs. These experiments were entirely negative and, consequently, lend no support to the infection theory. The same investigators tested the infectious nature of the disease by injecting puppies with blood recently drawn from rachitic children. These treatments had no effect upon the health or growth of the animals; indeed, it is stated that finer puppies were never seen in the laboratory. Some one has suggested the idea that rickets might be due to a virus transmitted by body lice. However, this suggestion may be dismissed, because it is well known that children who have always been free from body lice may have and do have rickets. Findlay calls attention to the fact that he found flea bites more freely in rachitic than in nonrachitic children. In his own studies he observed that without exception rachitic children were flea bitten, often badly, but that nonrachitic children were not bitten at all. This, however, he is inclined to attribute to the poor parental care and bad hygienic conditions under which his rachitic children lived, and he does not believe that the flea bite is a factor in the etiology of the disease.

(3) *Deficiency of Fat in the Diet Theory*.—Along in the eighties Bland-Sutton stated that rickets would appear in young animals if they were wholly deprived of milk, whatever the diet might otherwise be, but, almost immediately following this announcement, Baxter showed that Bland-Sutton's animals had not developed rickets, but marasmus. Furthermore, the same investigator saw rickets develop in his control animals fed upon milk and oatmeal. The last mentioned investigator had among his controls one puppy which he intended to give to a friend and on account of his special interest in this animal, he frequently gave it exercise, while the others were kept in their kennels. He observed that all appearances of rickets in this animal vanished after a long



period of exercise. This set Baxter to thinking and he took a litter of puppies, divided them into two lots, both were fed upon the same diet, consisting of milk, porridge, and horse flesh, all were housed in the same room, some were confined in a cage while others were allowed freedom and on occasions were taken out for walks. The confined animals became markedly rachitic, while those allowed their freedom did not.

(4) *The Vitamin Theory*.—Mellanby, at the University of Cambridge, has for the past few years been engaged in a series of experiments on the etiology of rickets. He has convinced himself that this disease is due to a diet deficient in a vitamin in some way connected with the fat of the food. He does not think that the disease is due to the absence of fat as fat, but because there is linked to the fat an antirachitic vitamin identical with or similarly connected with the fat, as is the case with fat-soluble A. Paton, of the Glasgow School, has contested this claim. Of two litters of pups in a metabolic study, five were sent to the country, being fed on a diet of porridge and skimmed milk. The daily intake of fat was not more than 2.8 grams. These animals had abundant exercise. The controls, kept at home in the laboratory, fed on porridge and whole milk, with an occasional addition of butter, so that the daily intake of fat, at first three grams, rose to six, and in some instances this increase in fat was carried to 14.5 grams. All the puppies kept in confinement, on being killed when eleven weeks old, showed signs of rickets, those receiving the greatest amount of fat being most severely affected, while those on a scant fat diet but exercised in the country, remained perfectly normal. It seems that Paton and his helpers have demonstrated that Mellanby's claims are unjustified.

(5) *Insufficient Exercise Theory*.—Findlay thinks he has evidence, both from experimental and from clinical sources, that the most potent factor in the causation of rickets is lack of exercise. We have already, under the preceding heading, given an illustration of the experimental evidence. Findlay and his assistants have studied the effects of environment, especially of housing, on rickets in Glasgow. They found that the average air space per person in families where marked active rickets was present was 396 cubic feet; in cases of slight rickets, 452 cubic feet, and in families where there were no rachitic children it was 565 cubic feet or more. Rachitic families were twenty per cent larger than nonrachitic and in the former the health of the mother was bad in nearly thirty per cent, while in the latter this was true in only five per cent. Diet did not seem to play any important rôle in the development of rickets. The average caloric intake per man in the rachitic families was 3315 and in the nonrachitic families, 3390. In the rachitic families twenty per cent consumed less than 3000 calories per day, while this held good for twenty-five per cent in the case of the nonrachitic. Recently Hutchison has studied the relation between housing and dietetic conditions and the prevalence of rickets among the rich and the poor in Nasik in India. It is the custom of wealthy Hindoos and Mohammedans in that region to confine their children in airless and sunless rooms, not only during infancy, but also in the case of girls until puberty or until marriage. On the other hand, the poor live in huts or tents and practically lead an outdoor life. The articles entering into the diets of the two classes



are the same in quality, but of course, different in quantity. There is practically no difference between the energy value of the diets of the two classes, but in the poor much less of this is represented by fat than in the case of the rich; and what is more important, in the diet of the rich the fat is present chiefly in the form of milk, whereas in the poor, animal fat is often absent from the diet altogether and if present, only in small amounts. Hutehison found early rickets during infancy and late rickets at more advanced ages prevalent among the rich and scarce among the poor. Moreover, he found that when the rachitic children of the rich were placed in the sun and fresh air, no change in diet being made, they recovered rapidly.

(6) *The Sunshine Theory*.—It is an interesting fact that Glisson, the first to observe this disease, called attention to its prevalence in dark, foggy places hidden away from the sun, and it grows more evident from all the experiments that are being made that sunshine is an important factor in the prevention of this disease. Of course, the adjuvants, proper diet and adequate exercise must not be forgotten.

—V. C. V.

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## OBITUARY

G. SIMS WOODHEAD

THOSE of us in America who have had the pleasure and honor of entertaining Dr. Woodhead in our homes have learned with deep regret of his untimely death in December, 1921. He was the son of a Yorkshire newspaper man and was born in 1855. He began his medical studies at the University of Edinburgh in 1873 and graduated in 1877. Soon thereafter he was appointed an assistant to the professor of pathology, in which position he soon attained distinction, both as an investigator and as a teacher. In 1883 the first edition of his splendid work on Practical Pathology was issued. Woodhead's success as a teacher and organizer in Edinburgh led to his being called to be the first superintendent of the Laboratory of the Royal College of Physicians in London. Here continued success was secured and in 1899 he was called to fill the very important chair of pathology at the University of Cambridge. In 1901 he became a member of the Royal Commission on Tuberculosis, and to his work on this Commission we are largely indebted for the facts which we now have establishing the responsibility of the bovine tubercle bacillus in the causation of human tuberculosis. It will be remembered that in 1901 Koch stated that in the warfare against tuberculosis the milk problem might be altogether neglected, since the bovine bacillus plays no part, or at least an unimportant part, in the causation of tuberculosis in human beings. Woodhead, in England, with his confreres, and Park, in this country, demonstrated beyond question that, in children at least, the bovine bacillus is an important factor in the causation of

tuberculosis and that we can by no means ignore the possibility, indeed the certainty, of the distribution of this disease through infected milk.

From early manhood Dr. Woodhead was an enthusiastic supporter of the Volunteer Medical Corps, and at the outbreak of the European War he was placed in medical charge of the Irish Command Depot in Tipperary. During the later years of the War he acted as consultant and as inspector of laboratories throughout the Kingdom. At the close of the War the tuberculous soldier received his special attention and he did not spare himself in the organization of sanatoria and colonies for the benefit of these men. He founded the *Journal of Pathology and Bacteriology*, was one of the leading supporters of the Pathological Society of Great Britain and Ireland, and was a member of the Executive Committee of the Imperial Cancer Research Fund. He also served on the Treasury Committee of the Scottish Universities, and his advice was generally sought throughout the British Empire. He was a total abstainer, and will be remembered not only for the scientific and practical work that he did, but for the cheer and good comradeship which he carried with him wherever he went.

—V. C. V.



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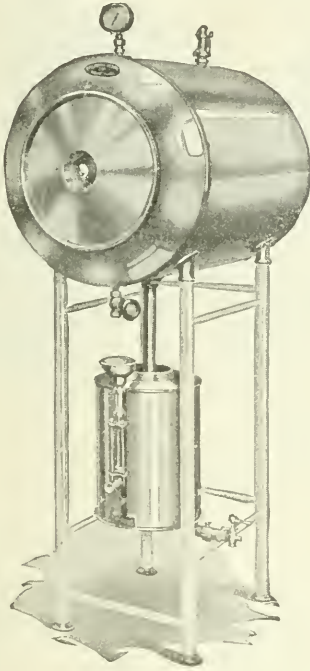
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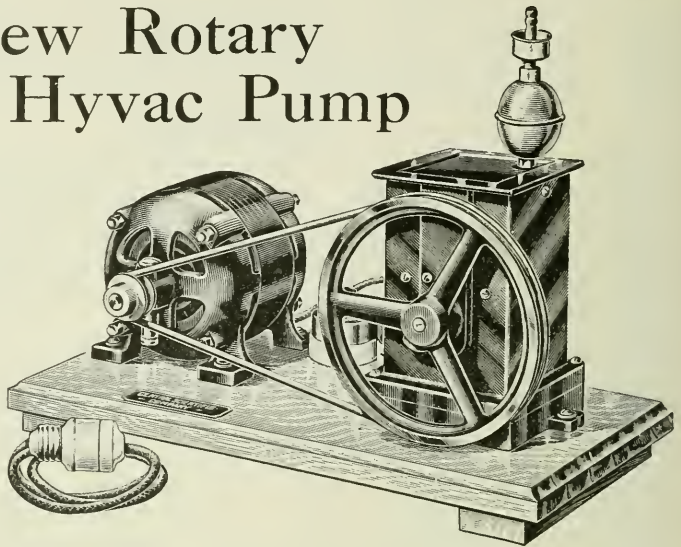
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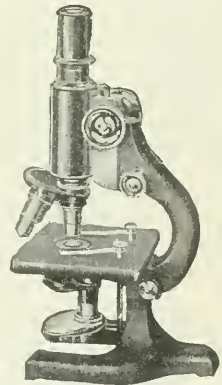
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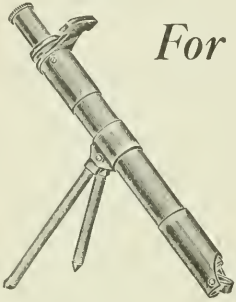
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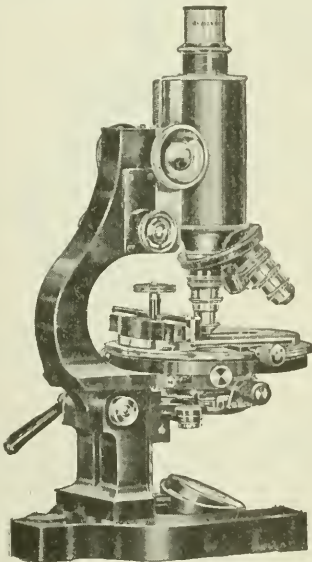
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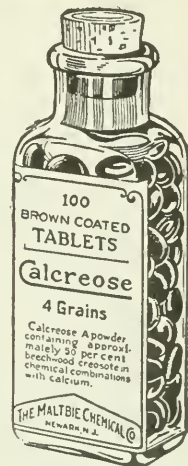
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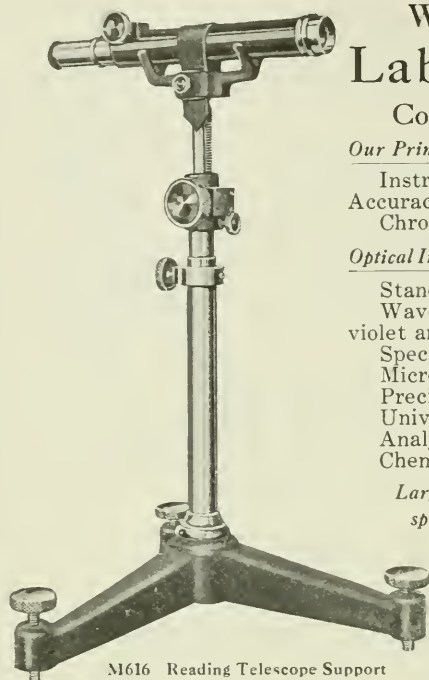
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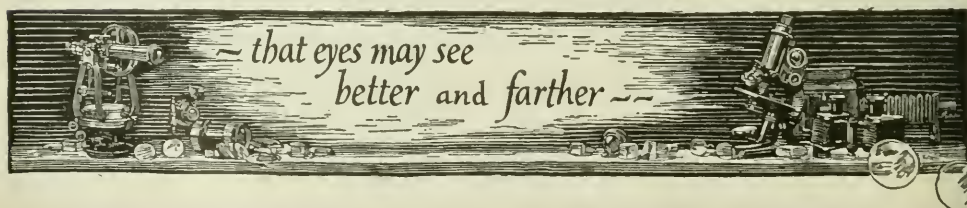
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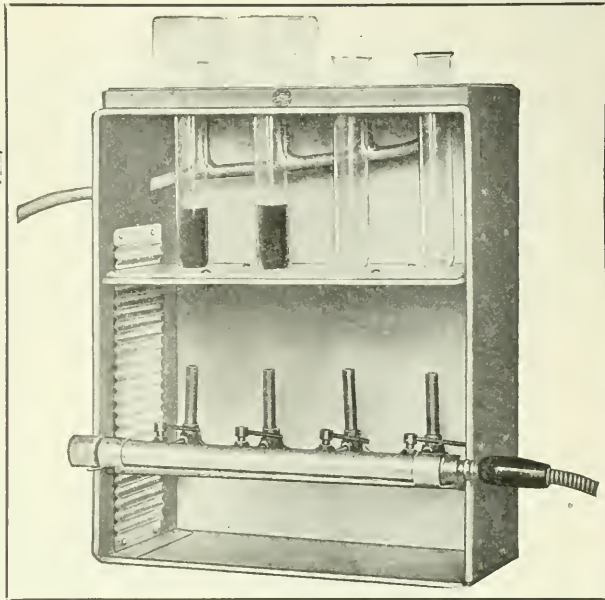
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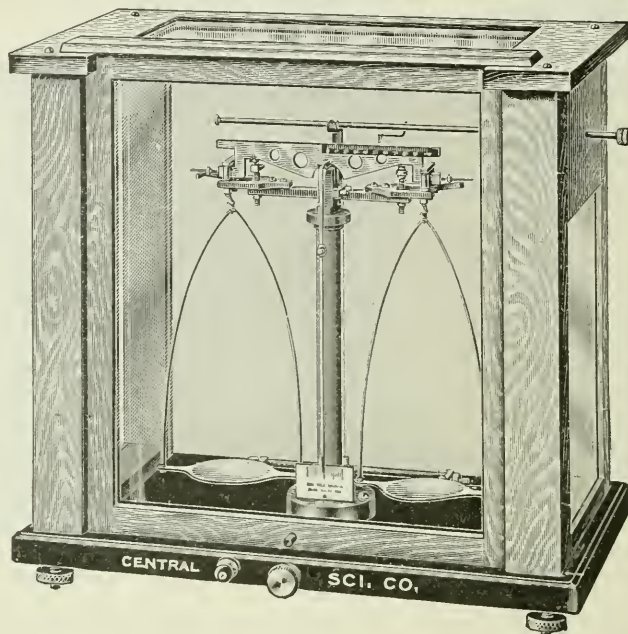
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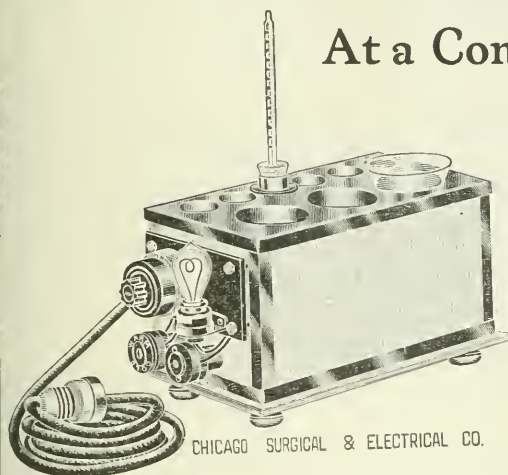
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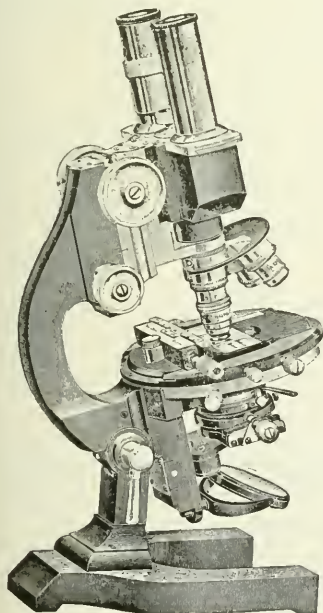
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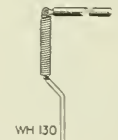
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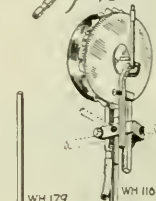
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# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, SEPTEMBER, 1922

No. 12

## ORIGINAL ARTICLES

### ELECTIVE LOCALIZATION OF BACTERIA FOLLOWING VARIOUS METHODS OF INOCULATION, AND THE PRODUCTION OF NEPHRITIS BY DEVITALIZATION AND INFECTION OF TEETH IN DOGS\*

BY EDWARD C. ROSENOW, M.D., AND JOHN G. MEISSER, D.D.S.,†  
ROCHESTER, MINN.

THERE are many facts which indicate that focal infections play an important part in the etiology of nephritis.<sup>14, 17</sup> Various forms of nephritis have been produced by intravenous injections of streptococci.<sup>15, 16</sup> Improvement, especially in acute and subacute nephritis, occurs often after removal of tonsils and other foci of infection.<sup>3, 6, 8, 11, 13</sup> The tolerance of the kidney to repeated injections of arsphenamine in the treatment of syphilis is greater in persons free from focal infections.<sup>9, 25</sup> The incidence and severity of nephritis and other complications in scarlet fever are far less in persons whose tonsils have been removed than in those in whom tonsillectomy has not been performed.<sup>4, 12</sup> The idea that advanced chronic nephritis is the result of repeated onslaughts and not the result of a continuous process has been recently emphasized.<sup>10</sup>

In the course of numerous experiments in animals lesions of the kidneys were found to occur more often following intravenous injection of streptococci from diseases in which involvement of the kidney occurs than from diseases in which the kidneys are spared.<sup>18</sup> Twenty per cent of the rabbits injected with *Streptococcus viridans* from chronic infectious endocarditis, a disease in which glomerulonephritis due to this organism is commonly

\*Presented before the American Society for Experimental Pathology at the annual meeting of the Federation of American Societies for Experimental Biology, New Haven, December, 1921.

Received for publication, January 14, 1922.

†Division on Experimental Bacteriology, The Mayo Foundation.

noted,<sup>2</sup> developed focal lesions in the kidneys. Lesions in the kidneys were noted in 39 per cent of the animals injected with the streptococci from rheumatic fever. The type of lesion in these was such that its repeated occurrence might ultimately result in a scarred contracted kidney, such as develops not infrequently in persons with recurring attacks of rheumatic infections.

During the course of numerous experiments on the intravenous injection of bacteria, especially relatively nonvirulent laboratory strains of streptococci, lesions of the kidney did not occur until after the virulence was increased by animal passage. The affinity for the kidney became greater after the streptococci from appendicitis, ulcer of the stomach, and cholecystitis were passed through a number of animals.<sup>19</sup>

These observations suggest that if focal infections play an important part in the production of nephritis, the foci in these cases may harbor bacteria which have a predilection for the parenchyma of the kidney. This has been found to be the case following the intravenous injection of bacteria from foci of infection in four selected cases of nephritis, representing different types of the disease.<sup>20</sup> Of the thirty-three animals injected with the primary cultures, twenty-seven (82 per cent) developed lesions in the kidneys. The affinity of the streptococci isolated from the lesions in the kidneys of these animals (second animal passage) was also marked, while in the third animal passage it had largely disappeared. The incidence of lesions in other organs was small. The lesions following injection of the strains from chronic nephritis were relatively slight as compared with those following the injection of bacteria from acute nephritis. A selective survival in the parenchyma of the kidney of streptococci having affinity for this organ was also demonstrated. The conditions and type of lesion noted commonly in pyelonephritis have been reproduced in the main by Bumpus and Meisser following intravenous injection of freshly isolated strains of streptococci from dental and other foci of infection in patients with pyelonephritis.

It must not be assumed that it is essential to inject the bacteria intravenously in order that they may localize in the tissues for which they have elective affinity. Characteristic localization has been obtained repeatedly following other methods of inoculation. Marked ulceration of the stomach in guinea pigs occurred following intraperitoneal injection of the streptococcus from a suppurative sinus from an infected tooth in a patient with acute ulcer of the stomach. Suppurative pulpitis and hemorrhagic edema of the periosteum opposite the roots of teeth in animals followed the intraperitoneal injection of the streptococcus from the pulp of a tooth of a patient who had recurring attacks of pulpitis, dental neuritis, and myositis.<sup>21</sup> Localization in the alveolar epithelium of the lungs in guinea pigs occurred after intraperitoneal injection of the highly virulent green-producing streptococcus from influenza.<sup>22</sup> Suppurative endometritis was found often after intratracheal inoculation of this organism, freshly isolated from sputum or lung exudate in cases of severe pulmonary influenza during the epidemic in 1918.<sup>22</sup> Localization of streptococci, with leukocytic infiltration, was found in the ileum following the intratracheal injection of a small amount of hemorrhagic mucus con-



taining streptococci from a patient with intestinal influenza; hemorrhagic colitis developed after the freshly isolated streptococcus, in oil suspension, from another patient with intestinal influenza was injected into the stomach.<sup>23</sup>

The extreme specificity for muscle tissue of a streptococcus from a case of myositis was shown in an experiment in which the organism injected intravenously not only localized in the muscles of a pregnant rabbit, but also in the corresponding muscles of the fetuses.<sup>24</sup> A streptococcus isolated from a tooth of a patient with recurring attacks of iritis, myositis, and arthritis produced iritis, myositis, and arthritis on intravenous injection, and also produced iritis, including the uninjected eye, and lesions in muscles when extremely small doses were inoculated into the anterior chamber of one eye.<sup>20</sup>

In the light of these striking results there is no reason to doubt that specific localization in distant tissues or organs may occur from chronic, localized, often symptomless, infections. It has been shown that the bacteria are in close proximity to the newly forming blood vessels in chronic foci of infection, such as granulomas at the apices of devitalized teeth, and are not incapsulated in scar tissue.<sup>24</sup> The fundamental requirements of the principles involved in the production of lesions remote from a focus of infection have been fulfilled, but in order to test the effect of one type of chronic focus of infection, produced so frequently in dental practice through the devitalization of teeth, teeth in dogs were devitalized and infected with bacteria having elective affinity for various tissues.

#### THE RESULTS OF EXPERIMENTS WITH A STAPHYLOCOCCUS HAVING ELECTIVE AFFINITY FOR RENAL TISSUE FROM A PATIENT WITH NEPHRITIS: HISTORY OF PATIENT

CASE A 334085. Mr. R. S., aged twenty-one years, came to the Clinic November 8, 1920, with a history of having had repeated attacks of acute nephritis for six years. In a number of these attacks his condition had been critical. Partial recovery had ensued after prolonged rest in bed, a salt-free and protein-low diet, and the usual elimination treatment by sweats and cathartics. In several attacks he had had marked generalized edema, weakness, shortness of breath, headache, nausea and vomiting, and large amounts of albumin and many casts in the urine. On one occasion the urine contained much blood. He had been subject to frequent attacks of "cold in the head" as long as he could remember. Several acute attacks and less severe exacerbations of his renal condition followed nasopharyngeal infections.

Examination revealed a dilated heart and moderate edema of the lower extremities. The systolic blood pressure ranged from 165 to 190, the diastolic from 110 to 140. The hemoglobin was 42 per cent, the erythrocytes 2,460,000. The leukocytes on four occasions were 4,600, 6,400, 2,400 and 15,000. A Wassermann test of the blood, and two blood cultures were negative. The eye grounds were normal. The urine contained a large amount of albumin, and a moderate number of hyaline casts, renal cells, erythrocytes, and leukocytes. The phenolsulphonephthalein test revealed marked impairment of renal function. The blood urea and creatinin were extremely high. A detailed study of the blood chemistry will be reported by Dr. Rowntree, to whom we are indebted for the opportunity of studying this case.

Careful search for foci of infection revealed a cloudy left antrum, inflamed mucous membrane of the nose, especially on the left side, and moderate sized red tonsils from which liquid pus could be expressed. The teeth were normal. Owing largely to the results of the animal experiments reported herein, tonsillectomy was advised and performed, and the

left antrum was irrigated. Following these operations improvement was noted. The attacks of cold in the head were less frequent. Fewer casts, erythrocytes, and leukocytes were found; albumin in the urine became less, the blood urea diminished, and a moderate degree of health and strength returned. The patient's complete recovery appears out of the question because of the long duration of the disease and the probable extensive destruction of the kidney.

Cultures from the mucous membrane of the nose and from the washings of the left antrum yielded countless numbers of staphylococci in almost pure culture, while cultures from the emulsion of the tonsils yielded an unusually large number of staphylococci, together with hemolytic and green-producing streptococci. The effect of intravenous injection of the primary culture in glucose-brain broth (3 to 5 c.c. for adult rabbits) was studied in all in thirty-two rabbits. The affinity of the staphylococcus for the kidneys was marked. The streptococci failed to localize and were lost in the first animal passage. The percentage incidence of lesions which occurred in the various organs, in the first and subsequent animal passages, may be seen in Table I.

TABLE I

	ANIMALS INJECTED	PERCENTAGE INCIDENCE OF LESIONS IN						
		KIDNEYS	BLADDER	MUSCLES	JOINTS	MYOCARDIUM	PERICARDIUM	STOMACH
When isolated	11	82	9			18		
First, second, and third animal passages	11	100		27	55	27	9	9
Fourth, fifth, and sixth animal passages	10	100		40	10	80	20	20

Of the eleven rabbits injected with the primary cultures directly from the patient, six were injected with cultures obtained from the left antrum, and the mucous membrane of the nose, three with cultures obtained from the tonsils, and two with a suspension, in salt solution, of pus expressed from the tonsils; all except these two developed marked lesions in the kidneys. The incidence of lesions in other organs in the first animal passage was relatively slight. On successive animal passage, however, the incidence of lesions in other organs increased even though the organism retained its specificity for kidney tissue. A catheterized specimen of urine obtained before injection from each of the thirty-two rabbits was found to be normal in twenty-nine. Of the three remaining, one showed a trace of albumin, one a trace of albumin with a few pus cells and erythrocytes, and the other a moderate amount of albumin and a moderate number of erythrocytes in the urine. The bladders of twenty-five rabbits at necropsy contained sufficient urine for examination. In one of these the urine was normal, in twenty it contained albumin, a large amount in four instances, a moderate amount in sixteen, in four only a trace was present. Pus cells were found in the sediment in twenty-four, relatively few in twenty-one, a larger number in three. Usually there were no casts or erythrocytes, and if they occurred, were few in number.

The staphylococcus produced a yellowish growth on blood-agar and Loeffler's medium. Morphologically it resembled *Staphylococcus aureus* of furunculosis, but its virulence was relatively low. Of the thirty-two rabbits

injected fourteen died in from one to seven days. The others were chloroformed for examination in from three to seven days after injection.

On microscopic examination of sections of the kidneys of the rabbits injected intravenously large areas of focal necrosis, chiefly in the medulla, surrounded by dilatated vessels, were the striking picture, although sometimes small hemorrhages with necrosis of cells and leukocytic infiltration were marked features. Numerous small areas of slight leukocytic and round-cell infiltration were found between the tubules in the cortex. Besides these sharply localized lesions larger areas were found, often involving one or more medullary rays, in which there were marked necrosis of interstitial capillary endothelium, leukocytic infiltration between tubules, and swelling and non-staining of nuclei of tubular epithelium (Fig. 1). In the medulla corresponding to these areas the tubules, especially Henle's ascending limb, were often

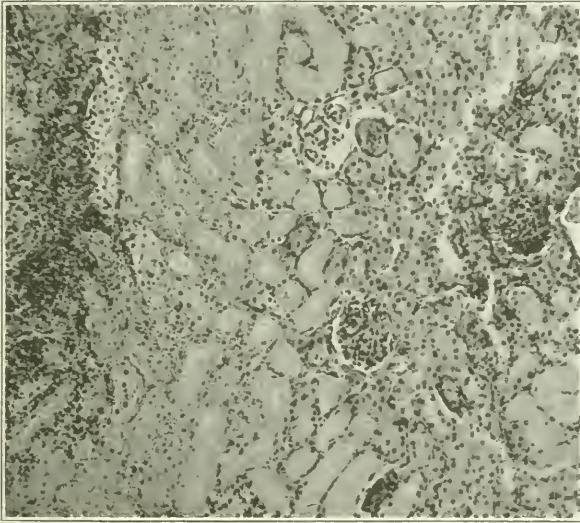


Fig. 1.

Fig. 1.—Section of kidney of rabbit (Rabbit 391) injected intravenously five days previously with the staphylococcus. Area of marked interstitial infiltration (left), adjacent area of swelling and non-staining of nuclei of tubular epithelium, and the area of normal kidney parenchyma (right). Hematoxylin and eosin (X 100).

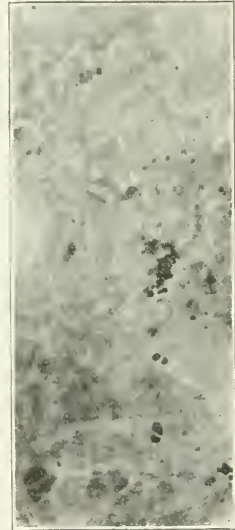


Fig. 2.

Fig. 2.—Staphylococci in area of intense interstitial infiltration shown in Fig. 1. Gram-Weigert (X 1000).

filled with varying proportions of degenerated and desquamated epithelium and leukocytes. The glomeruli were usually spared except as secondarily involved from intense infiltration of surrounding tissue. Bacteria were readily demonstrable (Fig. 2) in the different lesions within forty-eight hours after injection; after that the demonstration of bacteria was difficult, and sometimes it was not accomplished in the areas of diffuse infiltration.

About four months and about six months after the removal of the tonsils and irrigation of the antrum the staphylococcus was again isolated from the nasal mucous membrane and again produced lesions in the kidneys of rabbits on intravenous injection, but these were relatively slight. The affinity



for renal tissue of this organism, isolated from the tonsils and nasal mucous membrane in the first series of experiments, and the lesions on intravenous injection, were so marked that it was thought to be a favorable strain with which to produce nephritis through a chronic focus of infection.

Pure cultures in glucose-brain broth of the staphylococcus recovered from lesions in the kidneys of the rabbits were used to infect the pulp chambers of teeth in dogs. Active, well nourished, vigorous animals were selected. Catheterized specimens of urine from all were examined and found to be normal. In devitalizing and infecting the teeth the animals were covered with a sterile sheet, and kept under ether anesthesia by the intratracheal method. A rubber dam was applied to the mouth, isolating the teeth to be worked on. The teeth were scrubbed, and sterilized with alcohol and tincture of iodine, then cut off with sterile bone nippers midway between the incisal edge and the gum margin. The pulps of the two lower cuspids were carefully removed with sterile broaches, and after the hemorrhage had ceased the bacteria, in dense suspension, were introduced into the pulp chambers with a fine capillary glass pipette. The canal was then sealed with an impervious dental cement. The animals were numbered, kept under hygienic conditions, and fed a balanced diet of dog biscuit, with the occasional addition of meat, as were the control dogs. Catheterized specimens of urine were obtained and examined at intervals. Cultures, and gross and microscopic examinations were made of one kidney, which was removed by Dr. C. S. Williamson in from one to two months after the teeth were infected, so that the findings could be compared with those of the opposite kidney examined after a longer duration of the experiment.

Cultures from the kidney were made by inoculating blood-agar plates and glucose-brain broth with pipetted material after searing the surface of the kidney, and by placing partially macerated pieces of the kidney in glucose-brain broth. The localizing power of the bacteria isolated from the focus and from the lesions in the kidney was tested by intravenous injection into rabbits. Portions of the kidney showing lesions were fixed in 10 per cent formalin, and sections were mounted in paraffin and stained with hematoxylin and eosin to determine the character of the lesions, and by the Gram-Weigert method for the demonstration of organisms.

December 10, 1920, the pulps of the two lower cuspids were removed from Dog 2, and the pulp chambers infected with the staphylococcus from the lesions in the kidney of one of the rabbits injected with the culture from the washings of the left antrum of the patient. There was no swelling or tenderness of the dog's jaw following the devitalization.

January 5, 1921, the animal appeared well. A catheterized specimen of urine contained leukocytes, a few erythrocytes, a trace of albumin, and calcium oxalate crystals. The right kidney, which was removed, appeared normal on the surface, and the capsule stripped readily. The cortex was normal. In the medulla a number of small radiating elongated areas of marked hyperemia were found. Cultures of this kidney remained sterile. Microscopic examination revealed a number of small areas of hemorrhage with slight leu-



kocytic infiltration in the cortex, a number of small areas of necrosis in the medulla, with little or no cellular infiltration, and one larger area of focal necrosis in the medulla with marked leukocytic and round-cell infiltration and degeneration of the tubular epithelium. A few staphylococci were found in this area; in the smaller areas of necrosis and hemorrhage, bacteria were not demonstrated. February 12, the dog died and was examined within an hour. It was emaciated. The devitalized teeth were markedly discolored, but firm, and the cement was in proper place. There was no purulent exudate in the nose. Examination of the lungs revealed small areas of bronchopneumonia and several small abscesses filled with yellowish pus. Smears of the pus contained large numbers of staphylococci. The pleural cavities were empty, and the pleura was smooth. There were no lesions in the stomach, liver, gall



Fig. 3.—Kidney of Dog 2 showing widely disseminated areas of focal nephritis.

bladder, urinary bladder, muscles, heart, joints, or nerve trunks. The remaining kidney was swollen. Numerous whitish areas were seen through the capsule, particularly in the upper two-thirds. The capsule was adherent opposite a number of these areas, but otherwise stripped readily. The cut surface of both the cortex and the medulla presented large and small grayish white, irregularly shaped areas, and streaks with intervening portions of more normal renal tissue (Fig. 3). Some of these areas conformed to the size and shape of medullary rays and appeared almost necrotic, but in none had softening occurred.

On microscopic examination it was found that the larger whitish areas, especially in the cortex, consisted of intense interstitial endothelial hyperplasia and infiltration, with large cells, probably fibroblasts, small round cells, and plasma cells in variable proportions. The large mononuclear cells predominated over wide areas and in these many karyokinetic figures were found (Figs. 4 and 5). Some areas were clearly embolic in origin, the center being necrotic; the structure of the parenchyma was lost. In other areas no

central necrosis was found, but there was instead a more diffuse, widely disseminated infiltration in which the structure of the kidney was obscured. The infiltration often completely or partially surrounded blood vessels and malpighian corpuseles, but the capillaries in the glomeruli and the epithelium of Bowman's capsule were normal (Fig. 4) except that in some instances the cellular infiltration extended along the vessels as they pierced the capsule. In some areas connective tissue formation was marked (Fig. 6). Numerous small areas of interstitial infiltration were found, especially in the medulla, where the collecting tubules and the ascending limb of Henle's loop were often filled with degenerated, desquamated epithelial and other cells. These were usually found in direct line with the larger wedge-shaped areas of cellular infiltration in or near the cortex. The areas varied in size from that of an accumulation of a few leukocytes in the capillaries or tissue spaces between the tubules to

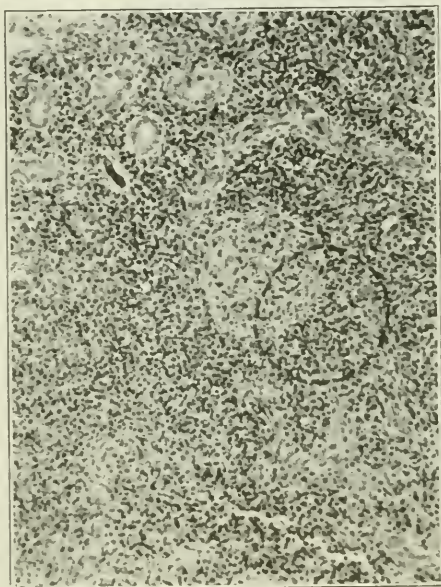


Fig. 4.—Section of kidney of Dog 2. Diffuse proliferation and infiltration by round cells, and beginning formation of interstitial connective tissue; glomerulus normal. Hematoxylin and eosin (X 50).

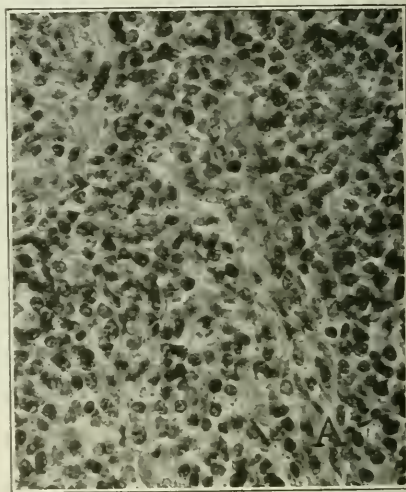


Fig. 5.—Higher magnification of area in small circle in Fig. 4, showing character of round cells and karyokinetic figures (a). Hematoxylin and eosin (X 500).

large areas in which many tubules were involved. In the more normal kidney structure only occasional areas of hemorrhage, necrosis, and infiltration were found. Prolonged search was necessary to demonstrate the presence of the staphylococcus in the lesions. No other bacteria were found.

Cultures from the kidney and the urine in glucose-brain broth yielded a pure growth of the staphylococcus. Those from the blood, bile, and spleen were negative. Cultures from the lung abscesses yielded staphylococci, streptococci, and *Bacillus bronchisepticus*.

A roentgenogram of the dog's lower jaw showed evidence of periapical rarefaction of the two devitalized cuspid (Fig. 7). On removing the alveolar process over the apex of each tooth a rarefied area 3 to 5 mm. in diameter was

broken into. The tissues were soft and edematous, and the pulp chambers were filled with a dark brown, thick fluid, in which large numbers of gram-positive cocci, often in clumps, streptococci, and small gram-negative bacilli and leukocytes were found. Blood-agar-plate cultures made from these areas showed staphylococci, green-producing streptococci and some gram-positive bacilli.

The localizing power of the staphylococcus isolated from the urine and kidney of this dog was determined by intravenous injection into rabbits. The two injected with the culture from the urine developed marked lesions of the kidney, and one slight lesions of muscles. One of the three injected with the kidney culture developed marked lesions in the kidney; the other two, slight lesions. The staphylococcus was isolated in pure culture from the kidney

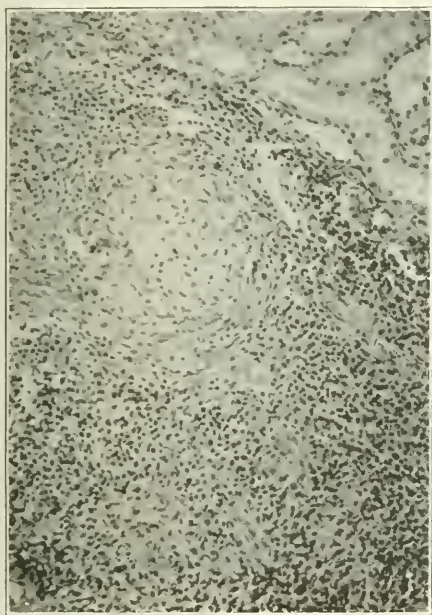


Fig. 6.—Section of kidney of Dog 2. Area of well advanced connective tissue formation surrounded by intense interstitial round-cell infiltration. Hematoxylin and eosin (X 100).

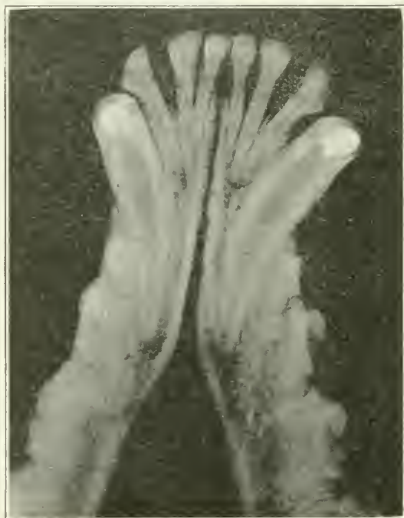


Fig. 7.—Roentgenogram of lower jaw of Dog 2, showing large rarefied areas at the apices of the two lower cuspids, which were devitalized and infected.

lesions in all of the rabbits. It was thought worth while to see whether the highly specific localizing power could be made to disappear by injecting the strain into a relatively insusceptible animal. Two guinea pigs were injected intraperitoneally. Both remained well, and were anesthetized for examination seventeen days after injection. An abscess containing thick pus was found in the peritoneum of each. Cultures of the pus yielded a pure growth of the staphylococcus. Two rabbits were injected intravenously with the primary culture in glucose-brain broth. Both remained well and were anesthetized in forty-eight hours. No lesions were found in the kidneys or other organs, and the strain was lost.

December 16, 1920, the pulps were removed from the lower cuspids of



Dog 3, and the pulp chambers infected with the staphylococcus recovered from the lesions in the kidney of a rabbit injected with the cultures from the patient's tonsils.

January 4, 1921, the animal appeared well. A catheterized specimen of urine showed erythrocytes and leukocytes, but no albumin or casts. February 19, the animal had lost in weight. The right kidney was removed. The capsule was adherent opposite eight small whitish scars which on section appeared to be healed embolic areas of focal necrosis. Microscopic examination showed small areas of interstitial, round-cell infiltration between the tubules in the cortex, and in the glomeruli and immediately adjacent to them. The medulla was free from lesions. March 4, the animal was found dead. It had lost much in weight. The devitalized teeth were markedly discolored,



Fig. 8.—Bladder of Dog 6, showing calcareous deposit in adherent exudate.

but firm. The lungs contained a few atelectatic areas and a few patches of peribronchial consolidation. The stomach had a moderate number of hemorrhages in the submucosa. The remaining kidney was enlarged. The capsule stripped readily, and beneath it were found six small whitish areas of necrosis in various stages of healing. By inserting a pipette into the pelvis a large amount of thick grayish pus was obtained. The pelvis was filled with pus and a thick adherent membrane, in which numerous concretions were found. The mucous membrane was edematous and hemorrhagic. The cortex was swollen and grayish in color. The cortical markings were distinct. In the medulla there were a moderate number of linear grayish yellow streaks, surrounded by a hyperemic zone.

The left ureter was hyperemic and swollen, especially in the upper 3 cm., where numerous linear and punctate hemorrhages and an adherent membranous exudate were found. The right ureter also contained punctate



hemorrhages in the mucous membrane, but there was no evidence of infection where it had been severed from the kidney some weeks before.

The wall of the bladder was edematous throughout. The mucous membrane was hyperemic and had many areas of hemorrhage. Adherent to the mucous membrane were many nonlaminated, long, rough, pumice-like concretions which were grayish yellow and which radiated in a fan-like manner from the base of the bladder up along the blood vessels (Fig. 8). On chemical analysis these calcareous deposits were found to contain calcium oxalate and calcium phosphate. The urine contained many leukocytes and erythrocytes, and a large amount of albumin.

Sections of the kidney showed marked swelling of the epithelium of the convoluted tubules, and small areas of hemorrhage, necrosis, and infiltration in the medulla, and a few gram-positive cocci.

Smears of the pus from the kidney contained enormous numbers of gram-positive cocci, often in clumps, large gram-positive and gram-negative bacilli, and a few chains of gram-positive cocci. Cultures of the pus in the pelvis, of the urine, and from the devitalized teeth, yielded large numbers of staphylococci, gram-positive and gram-negative bacilli, and streptococci. The cultures of the blood and of the substance of the kidney remote from the pelvis yielded a pure growth of staphylococci.

One of the two rabbits injected intravenously with the staphylococcus from the parenchyma of the kidney developed lesions in the kidney, the other, abscesses in muscles. Both of the rabbits injected with the staphylococcus from the pus in the pelvis developed lesions in the kidneys. The two injected with the gram-positive bacillus from one infected tooth remained well and no lesions were found at necropsy.

December 30, 1920, the pulp was removed from the two lower cuspids of Dog 5, and the pulp chambers were infected with the staphylococcus recovered from the lesions in the kidney of a rabbit injected with cultures obtained from the mucous membrane of the nose of the patient.

January 4, 1921, a catheterized specimen of urine contained erythrocytes and leukocytes. March 26, the right kidney was removed, and four distinctly opaque, adherent, but healed, thickened areas of scar tissue in the cortex beneath the capsule were found. Beneath several of these were wedge-shaped scars running well into the substance of the cortex and of the medulla. The cortex was cloudy and distinctly swollen. In the medulla were found wedge-shaped areas of marked hyperemia and numerous very small whitish streaks radiating outward from the pelvis and papilla. Cultures made from the kidney yielded staphylococci. Microscopic examination of sections of the kidney revealed the fact that the small whitish streaks found in the medulla were due to interstitial infiltration by small and large round cells in which mitotic figures were easily demonstrable. In the cortex were numerous very small areas of interstitial infiltration, frequently surrounding malpighian corpuscles, with at times invasion of glomeruli along the blood vessels of the hilum.

April 20, the urine contained leukocytes and a trace of albumin. The

animal had lost much in weight and much of its hair, and had developed a corneal ulcer in each eye. April 25, it was anesthetized and examined for lesions. The remaining kidney had numerous very small grayish white streaks radiating from the papilla outward. These lesions were not so marked or so numerous as in the kidney removed at operation.

Cultures made from the urine yielded only staphylococci, from the kidney many staphylococci and an occasional short chained streptococcus. From the apex of the devitalized teeth many staphylococci and an occasional short chain of streptococci were recovered. Cultures made from the fluid in the anterior chamber of the dog's eyes yielded staphylococci and a few streptococci. Cultures from the other organs were negative.

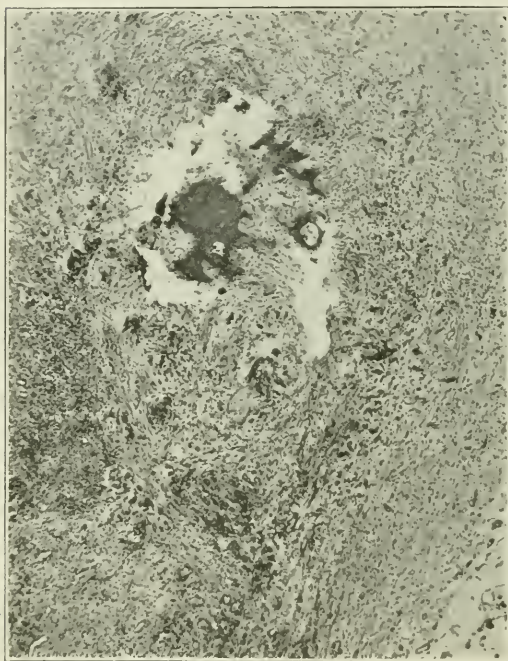


Fig. 9.

Fig. 9.—Photomicrograph of granuloma from apex of devitalized and infected tooth of Dog 5. Note the variations in the intensity of cellular infiltration in the connective tissue, and invasion of the bone. Hematoxylin and eosin (X 50).

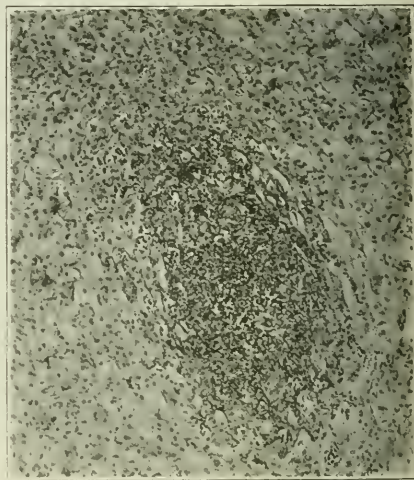


Fig. 10.

Fig. 10.—Photomicrograph of focal lesion in the medulla of kidney of Dog 6. Hematoxylin and eosin (X 70).

Sections of the kidney showed a few small circumscribed areas of round-cell infiltration in the interstitial tissue of the medulla, and larger areas of highly cellular, newly formed connective tissue surrounding the larger blood vessels in the cortex. Bacteria were not found.

Sections of the granuloma at the apex of one of the teeth showed dense connective tissue in which numerous sharply circumscribed areas of intense and mild cellular infiltration were found (Fig. 9). These areas were most numerous at the periphery of the granuloma at the point of erosion of the bone, but were also found in the denser areas of leukocytic infiltration immediately adjacent to the newly forming blood vessels.

December 12, 1920, the two lower cusps of Dog 6 were devitalized and the pulp chambers infected with a pure culture of the staphylococcus obtained from the lesions in the kidney of a rabbit injected with the culture from the patient's tonsils.

January 4, 1921, the animal appeared well. A catheterized specimen of urine contained erythrocytes and leukocytes, and a trace of albumin. February 19, the right kidney was removed. The capsule stripped readily, and there was no evidence of lesions in the cortex. On the cut surface, with the aid of a magnifying glass, numerous exceedingly fine linear grayish yellow streaks were found in the medulla. These were most numerous in the papillae and appeared on cross section as grayish yellow punctate areas. Cultures made from the medulla by means of a sterile pipette yielded a pure culture of staphylococci. Sections showed a moderate number of small areas of necrosis in the medulla, with little or no cellular infiltration, and numerous small



Fig. 11.—Cocci in area of hemorrhagic edema in cortex of kidney of Dog 6. Gram-Weigert (X 1000).

collections of round cells between tubules higher up in the medulla and in the cortex, and adjacent to the malpighian corpuscles. There was moderate congestion of the capillaries and the glomeruli, and small areas of hemorrhage between the tubules in which leukocytes were found. Bacteria were not demonstrated. March 9, the dog had lost much in weight, and appeared sick: it was chloroformed for examination. In the remaining kidney were numerous exceedingly fine linear streaks throughout the medulla. On microscopic examination of sections these were found to consist of aggregations of leukocytes within capillaries and tissue spaces between the tubules, collections of leukocytes and desquamated degenerated epithelial cells in the collecting tubules and ascending limb of Henle's loop. A number of large areas of necrosis with marked infiltration were found in the distal portion of the medulla (Fig. 10). Small areas of interstitial hemorrhage and edema were



found in the cortex, and small infiltrations of leukocytes and round cells in the small vessels between the tubules. In these, staphylococci only (Fig. 11), usually singly, but occasionally in clumps, often within the capillaries, were found, whereas, in the larger areas of necrosis in the medulla staphylococci and streptococci were easily demonstrable.

Cultures of the substance of the kidney and of the urine yielded staphylococci and green-producing streptococci, those from the infected material from the devitalized teeth, the same organisms, together with gram-positive and gram-negative bacilli.

A roentgenogram of the lower jaw showed large areas of rarefaction around the roots of both cuspids. The teeth were firm, and the cement firmly in place. The pulp chambers were moist with chocolate-colored fluid; the tissues over the apices were edematous and the bone was spongy; a well formed granuloma, about 4 mm. in diameter, was found over the root apex of the right cuspid, but none was found over the left cuspid.

Pure cultures of the organisms isolated from the kidney and from the tooth were injected intravenously into rabbits. Two rabbits were injected with the staphylococcus recovered from the kidney. Both developed lesions in the kidneys. Four rabbits were injected intravenously with the green-producing streptococcus from the kidney. All remained well, and the organs were free from lesions. One rabbit was injected with a culture of staphylococcus obtained from the granuloma and apex of the tooth. This animal had lesions in the kidneys. A mixed culture of gram-positive bacilli and green-producing streptococci and staphylococci (gram-positive bacillus was the predominating organism) was injected into four rabbits. One developed slight lesions in the kidney, myocardium, and muscles; the other three were free from lesions.

#### DISCUSSION AND SUMMARY

Neither tenderness nor swelling of the jaw followed the devitalization of teeth. The devitalized teeth were firm, but markedly discolored. Evidence of infection in the periapical region of each was found at necropsy. In three quite firm masses of moist connective tissue resembling granulomas were found. In the others the softening and infiltration of the bony structure was more diffuse and connective tissue formation was less marked. The pulp chambers usually contained dark, turbid fluid, in which were many bacteria and but few leukocytes. The staphylococcus was recovered from the devitalized teeth in each dog at the end of the experiment, and the elective affinity for kidney tissue, as shown by intravenous injection into rabbits, was still present in the cultures from the two dogs in which this point was tested. In some of the devitalized teeth secondary infection by streptococci and bacilli had taken place. These organisms did not manifest a predilection for kidney tissue on intravenous injection into rabbits.

The etiologic relationship of the staphylococcus inoculated into the teeth to the lesions in the kidneys in these experiments seems established. It was isolated from both the focus and the kidneys. It manifested elective localizing power for the kidneys of rabbits when injected intravenously. It was demon-



strated in the lesions and was not demonstrable in the normal portions of the substance of the kidney. The lesions in three of the dogs were progressive; in one dog healing was well advanced. Lesions in other organs were slight or absent. In two dogs (Dogs 3 and 6) secondary infection of the lesions in the kidneys occurred. In the former, which showed marked pyelitis and cystitis, gram-positive and gram-negative bacilli, streptococci, and staphylococci were found. In the latter, which had interstitial lesions, especially in the medulla, streptococci and staphylococci were demonstrable. Lesions in the parenchyma of the kidney occurred in all four dogs. In three there were no lesions in the mucous membrane lining the pelvis, ureter, and bladder; in one (Dog 3) there were marked pyelitis and cystitis. The finding of marked calcareous deposits in the adherent exudate in the bladder of this dog emphasizes the importance of infection as a factor in the causation of urinary calculi.

Lesions in the kidneys of the character obtained in this experiment did not occur in seven dogs in which the corresponding teeth were devitalized and infected with organisms from sources in patients other than those with nephritis, or in dogs kept under the same conditions but in which the teeth were not devitalized.

The staphylococcus in this experiment was peculiar in that it had such marked affinity for the kidney on intravenous injection, greater than that found in other strains of staphylococcus. This property tended to disappear on successive animal passage on intravenous injection and was entirely lost in the pus in abscesses in the guinea pig following intraperitoneal injection, but was retained for months in the infected teeth and kidneys of the dogs.

The lesions were, in the main, focal in character, although large, widely disseminated areas were found, especially following the devitalization and infection of the teeth of the dogs. In these areas there were marked diffuse interstitial proliferation and infiltration by round cells, and in some marked connective tissue formation besides.

The defensive mechanism of the kidney against bacterial invasion has been found to be marked and to be chiefly cellular in character. Extensive interstitial infiltration not by leukocytes, but by large and small round cells, occurred in response to localization of the staphylococcus. Indeed in some instances (Dog 2) the microscopic picture resembled very closely the findings recorded as characteristic of acute interstitial nephritis.<sup>1,7</sup> The demonstration of the staphylococcus in these diffuse areas of infiltration was difficult, and yet the proliferation must have been incited by this organism since all gradations occurred between the more strictly focal lesions in which the bacteria were easily demonstrable and the diffuse areas. This finding suggests that the inability to demonstrate bacteria in certain forms of nephritis does not mean that they may not be the inciting agent in some instances. Striking as these findings in the kidneys were, the general picture in the dogs was quite different from that in the patient. The dogs had no edema, they lost markedly in weight, and the urine rarely contained a large amount of albumin or many casts. The lesions in the kidneys of the dogs suggest strongly that if the experiments could have been continued for a longer time dysfunc-

tion of the kidney more like that in the patient might have developed. It is, however, impossible to be certain whether the staphylococcus was the cause of the nephritis in the patient.

The experimental production of a systemic disease through the devitalization and infection of teeth in dogs with an organism having elective localizing power removes, it seems to us, all objections to the acceptance of the theory of focal infection and elective localization as an important factor in the etiology of disease.

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## ARSPHENAMINE—SOME FACTORS WHICH INFLUENCE ITS COLLOIDAL PROPERTIES\*

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THE pentavalent 3-nitro 4-hydroxy and 3-amino 4-hydroxy phenyl arsinic acids, occur in well-defined crystals, the former exhibiting a characteristic dimorphism. When reduced to the trivalent arseno stage the crystalline character of the pentavalent acids is suddenly replaced by a strongly gelatinous tendency. The arseno compound is amorphous, insoluble in water, incapable of recrystallization, and its soluble acid salts, of which the dihydrochloride arspenamine is best known, are resinous substances incapable of crystallization.

This suggests that the reduction is accompanied by a polymerisation of the molecules, forming aggregates with colloidal properties. The change in physical characteristics is accompanied by a marked increase in therapeutic activity, which makes the arseno compound one of the most indispensable agents in the treatment of protozoal infections, and its satisfactory preparation a matter of great importance to medicine.

The generally accepted formula for arspenamine is  $\text{HCl.R.As=As.R.HCl}$ , in which R represents the substituted phenyl residue. This corresponds to the analytical results, and to the general course of analogous reductions, but it does not seem to account for the sudden substitution of colloidal for crystalline properties on reducing the arsenic acid, or for the variations in these properties with different methods of preparation. It probably represents the structure of the arspenamine molecule reduced to its simplest terms, the extreme reactivity producing a polymerisation or association with the formation of large complexes. In this sense the formula for arspenamine may be written thus:  $(\text{HCl.R.As=As.R.HCl})_x$ . This would explain the generally colloidal properties by the size of the molecular aggregate.

The stimulus to the chemical investigation of arspenamine and its derivatives resulting from the granting of licenses for the manufacture in this country, has led to the introduction of several products under the generic name of arspenamine, and also to the proposal of various preparative methods calculated to improve the product chemically, biologically or economically. The decided differences in the readiness with which various preparations dissolve in water has often been noted without explanations being offered. The originally imported Salvarsan was a greenish yellow amorphous powder which gave a clear solution with methyl alcohol. When just moistened with water it

\*Read before the Spring Meeting of the American Chemical Society at Rochester, N. Y., April, 1921. By courtesy of the Journal of Industrial and Engineering Chemistry.

From the H. A. Metz Laboratories, Inc., Brooklyn, N. Y.

Received for publication, April 17, 1922.



gelatinized, but when sprinkled on the surface of a large amount of water at ordinary temperature it dissolved readily and completely. The first brand of American made arsphenamine was practically insoluble in methyl alcohol, and when added to water of room temperature, formed gelatinous lumps which required heating or boiling to effect solution. In 1919, Kober<sup>1</sup> proposed a modified preparative method which yielded a product insoluble in methyl alcohol and which formed a jelly with water of room temperature. Recently Christiansen<sup>2</sup> has prepared arsphenamine which he states forms a jelly with cold water, and subsequently reported a change in the preparative method which yields a more readily soluble product. Some of the preparations which will be described in this paper, are apparently insoluble in methyl alcohol and when added to water of ordinary temperature form stiff jellies. Others dissolve readily and completely in methyl alcohol, and quickly form thin solutions with water of ordinary temperature.

The possibility of differences in the chemical identity of arsphenamine preparations has been considered, and although at times suggested by the striking differences in their characteristics, it seems very remote. Nitrophenolarsinic acid is in every case the starting point for the reduction to the arsphenamine base and this acid is a very distinct chemical individual. The various methods of reduction and conversion into the dihydrochloride which have been used, are of such nature that chemical rearrangements are probably excluded. The products may therefore safely be assumed to be "structurally" identical, and this claim is made for all commercial arsphenamines as well as for the products of some of the proposed variations in method.

It is a well-known fact that in the generally adopted syntheses of arsphenamines, chemically pure products can only be assured up to the point of the arsenic acids. Both the base and the dihydrochloride of the arseno compound are thrown out of solution in an amorphous condition and cannot be recrystallized. The impurities which occur, however, seem to be neither of a character nor in a sufficient quantity to produce the obvious differences which are found in the final products.

An alternative explanation which remains for these variations is on the physical grounds of molecular condition; they consist in differences in physical state, not chemical form. These in turn may be produced by differences in the method of preparation, and the work of which this paper is a report, consists in varying the process and noting the effect of different factors on the character of the end product.

A study of the literature shows that a large number of different procedures have been used for the preparation of arsphenamine. The method which has come into extended use for its production on a large scale consists in the reduction of the nitrophenolarsinic acid by means of sodium hydrosulphite, conversion of the resulting arseno base into its dihydrochloride in methyl alcoholic solution, and precipitation by means of ether or acetone. This is the method described originally by Ehrlich.<sup>3</sup> It has been generally adopted on account of the comparatively complete descriptions of the process which are available, and because of the smoothness of the one stage reduction, convenience of



manipulations, ready recovery of solvents and precipitants, and generally satisfactory nature of the products and yields. When suitably conducted it leads to an arspenamine identical with that prepared by the original manufacturers, readily soluble in water of room temperature with a minimum of gelatinous tendency and soluble also in methyl alcohol.

Practically all other methods of preparation which have been used are either suggested or described or covered in patents by Ehrlich and his collaborators in this field.

Nitrophenolarsinic acid may be reduced to the arseno compound either progressively, with the formation of intermediate reduction products, or directly in one step, with simultaneous reduction of the nitro and arsenical groups.

The progressive reduction depends on the use of selective reducing agents. Reduction of the arsenical group without affecting the nitro group may be accomplished by phosphorus and hypophosphorous acid, which act specifically. This reduction is described in a patent of the year 1914.<sup>4</sup> Calculated quantities of stannous chloride also reduce the arsenical group leaving the nitro group intact, according to a patent issued in 1914.<sup>5</sup>

For the specific reduction of the nitro group, forming the amido-phenol-arsinic acid, the following methods have been described:

- 1) By sodium amalgam in methyl alcoholic solution.<sup>6</sup>
- 2) By sodium hydrosulphite in cold solution, avoiding excess of reducing agent,<sup>7</sup>
- 3) By ferrous hydroxide in alkaline medium,<sup>8</sup>
- 4) Ammonium sulfide has been employed but has the disadvantage of producing a combination of sulphur in the arsinic residue, which must be subsequently removed.<sup>9</sup>

The simultaneous reduction of both nitro and arsenic groups in one step may be accomplished by sodium hydrosulphite as already described, or by hypophosphorous acid, using potassium iodide as a catalyst, as described in a patent of the year 1914.<sup>10</sup> It has also been claimed that zinc and concentrated acids in presence of sulphurous acid produce a simultaneous reduction of both groups, which stops at the arseno stage.<sup>11</sup> An electrolytic reduction was described by Barth in 1914.<sup>12</sup> Besides these methods, an interesting synthesis was described by Kahn in 1912,<sup>13</sup> which depends on a condensation of two reduction products of nitrophenolarsinic acid. When molecular solutions of aminooxyphenylarsine and aminooxyphenylarsenoxide are mixed, one molecule of water is eliminated and the arseno compound is formed directly.

Methods of isolating the dihydrochloride of the arseno compound are less numerous than those described for the reduction of the arsenic acid. There are in fact only two general methods, both described in the older literature. The first and generally adopted procedure consists in forming a solution of the dihydrochloride by adding the calculated amount of hydrochloric acid to a methyl alcohol suspension of the base, and precipitating the dihydrochloride from the solution by means of ether, acetone, or other anhydrous precipitants.<sup>14</sup> The second method is described in a patent of the year 1914;<sup>15</sup> and depends on

the insolubility of the dihydrochloride in an excess of aqueous hydrochloric acid. Here the aqueous solution of the reduction product is run into a large volume of aqueous hydrochloric acid, and the dihydrochloride may be obtained without preliminary isolation of the base, and without the use of organic solvents.

A number of these preparative methods have been applied recently to the laboratory production of arsphenamine by Kober,<sup>16</sup> Fargher and Pyman,<sup>17</sup> and Christiansen,<sup>18</sup> leading to products with variable physical characteristics.

With a view of comparing as comprehensively as possible, the effect of various methods of preparation in the properties of the final products, a series of experiments was laid out, covering 17 different arsphenamine preparations. The methods selected probably include all the essential variations in the known and practically applicable processes. Six of the above mentioned reduction procedures and the condensation process with arsine and arsenoxide were carried out. From these reduction products the dihydrochloride was prepared both by the methyl alcohol ether method, and by precipitation from aqueous hydrochloric acid.

In addition, some of the preparations made by the alcohol ether method were reprecipitated from aqueous hydrochloric acid, and some of the products obtained originally by the latter method were reconverted into the arseno base and then into arsphenamine by precipitation from ether.

It was observed, that arsphenamine prepared from the base by dissolving in a methyl alcohol solution of the calculated amount of hydrochloric acid, and precipitating by means of ether, was readily soluble in methyl alcohol, and also dissolved quickly in water, giving a thin limpid solution. The same arseno base, brought into aqueous solution as dihydrochloride, and precipitated from a large excess of aqueous hydrochloric acid, gave an arsphenamine which was insoluble in methyl alcohol and with cold water formed a jelly. One apparent exception to this rule was observed, in which precipitation from ether did not decrease the gelatinous nature of the arsphenamine, but this may have been due to certain other experimental factors. In general, the experiments show the behavior indicated above, with only variations in degree of difference between the two products. If an arsphenamine which dissolves readily in water of room temperature giving a clear thin solution, be reprecipitated by pouring its solution into an excess of aqueous hydrochloric acid, the resulting product no longer dissolves readily in water, but forms a viscous jelly. It also differs from the original by being insoluble in methyl alcohol. On the other hand, the difficultly soluble product, when reconverted into its base and then into the dihydrochloride in methyl alcohol, may be precipitated by ether, yielding a product readily soluble in water and methyl alcohol. This indicates that precipitation from aqueous solutions is a factor in increasing the gelatinous nature of arsphenamine, as compared with the material precipitated from anhydrous solvents. In other words the use of electrolytes for solution and precipitation increases the colloidal characteristics. This corresponds to one of the most striking properties of suspensoid sols, which are unstable in the presence of electrolytes. In certain concentrations the electrical charges of the suspensoid are neutralized

by the oppositely charged ions of the electrolyte, producing coagulation. There is a close parallel between this observation and the behavior of the arspenamine solutions. Aqueous solutions of arspenamine may be of the nature of suspensoid sols. When they are precipitated from solutions containing a large number of ions the neutralization of their surface charges produces coagulation or polymerisation which increases the gelatinous character of the dried product. These ionic effects are absent when non-ionized alcohol solutions and ether are used and the resulting products show a minimum of colloidal character. This explanation is offered tentatively to account for the wide variations in the physical characteristics of the different products described in this paper. Other possibilities must be considered on the basis of further study, which is being carried on. There is no doubt that other factors may also influence the character of arspenamine, such as temperatures, purity of reagents, amount of mineral acid, mechanically retained moisture and impurities.

The above observations deal exclusively with variations in the characteristics of the solid arspenamine and indicate their colloidal nature. The fact that arspenamine solutions exhibit the characteristics of colloid sols has been noted recently. Bauer, in 1919, <sup>19</sup> using Bechhold's ultra filtration method, found that an alkalized Salvarsan solution, after standing for some time, left a considerably smaller amount of residue on the filter than did the freshly prepared solution. This corresponds to the gradual decrease in the size and number of colloidal particles called peptization. The same author found that solutions made from commercial "Salvarsan Sodium," which is produced by precipitation from anhydrous media, left no residue when passed through the ultra filter.

Klemensiewicz, in 1920, <sup>20</sup> found that aqueous arspenamine solutions exhibited viscosity phenomena which ranged them among the emulsoid colloids.

These physicochemical observations on the colloidal characteristics of arspenamine solutions, as well as the variable characteristics of the dry arspenamine described in this paper, appear to have a direct bearing on some of the biological and clinical observations which have been made in recent times. There have been noted wide variations in toxicity to experimental animals, sudden isolated cases of untoward clinical reactions from products which in the great majority of cases gave no trouble, as well as the toxic differences in different preparations. The large amount of purely chemical work which has been devoted to the question of the variability of arspenamine has been singularly lacking in definite results. Although arspenamine is not strictly speaking a chemically pure substance there is no known chemical impurity with the presence or proportion of which its toxic variability can be brought into connection. The only definitely recognized chemical substance to which extra toxicity can be ascribed is the so-called arsenoxide, resulting from the oxidation of arspenamine. Ehrlich<sup>21</sup> first suggested the higher toxicity of this compound and the danger of its presence. Since then however, the consensus of opinion is that arsenoxide is neither so readily formed nor as highly toxic as was originally supposed. As a matter of fact, arsenoxide has never been demonstrated to be present in arspenamine by actual isolation. This failure



of chemical investigation to bring to light a definite chemical impurity whose presence causes variations and extra toxic effects in arsphenamine, is of course not a proof of its absence. The symptoms shown by biological experiments on animals seem to indicate that the toxic effects are of different types.<sup>22</sup>

On the other hand, certain biological observations seem to correspond well with the results of physicochemical examinations of arsphenamine solutions. G. C. Lake found a decided decrease in the toxicity of alkalinized solutions on standing. A solution which was injected immediately after preparing, produced 14 fatalities in 15 rats injected in dosages of 120, 130, and 140 mg. per kilogram, 5 at each dosage. The same solution after standing for 30 minutes, produced only 2 fatalities at the same dosage and in the same number of animals. This almost inevitably suggests a connection with the results observed by Bauer, referred to above, in which he found the amount of colloidal particles in an alkalinized solution to decrease on standing. The recently communicated experiments of Reid Hunt,<sup>23</sup> who found that he could greatly reduce the toxicity of some arsphenamines by gently warming, or allowing the solutions to stand may also be explained by a so-called peptization of colloidal particles.

Whether or not these colloidal properties of arsphenamine cause all or any of the variations and untoward effects of arsphenamine can only be definitely demonstrated by systematic coordination of physicochemical and biological research. But there is already sufficient evidence at hand to indicate a connection between the physical state of arsphenamine and its solutions, and its biological effects. The study of the differences in the state of dispersion of various arsphenamine solutions, their dependence on the type of product used, on the method of solution and alkalinization, on the age of the solutions, and the changes in the disperse state produced on introduction into the blood stream, seems at least as promising as further work along purely chemical lines, to explain the phenomena connected with the preparation and use of arsphenamine.

#### EXPERIMENTAL

##### *Reduction I. Simultaneous reduction by sodium hydrosulphite.*

This was carried on in the usual way, essentially as in the original description of the method.<sup>3</sup> 31.5 gm. of the nitro-phenolarsinic acid yielded 42 gm. of slightly moist arseno base. 28 gm. of this base was dissolved in 150 c.c. of methyl alcohol by the addition of 2.6 gm. of 100% hydrochloric acid in methyl-alcoholic solution. The resulting solution of the dihydrochloride was filtered, and divided into two equal parts, from which the arsphenamine was precipitated as follows:

*Preparation No. 1.*—One-half of the solution was precipitated by 900 c.c. of anhydrous ether, the precipitated arsphenamine filtered, washed with ether, and dried in vacuo. Yield—7 gm. slightly greenish yellow powder. Arsenic—31.57 per cent. The product gives a clear solution in anhydrous methyl alcohol, and dissolves quickly in water of room temperature, forming a thin limpid solution when made up to contain 6 per cent of the dry substance.

*Preparation No. 2.*—The second half of the solution was run with stirring into 500 c.c. of hydrochloric acid of approximately 15 per cent, which had been cooled to 0° C. After standing for about 10 minutes, the precipitate was filtered by suction, washed with a small amount of ethyl alcoholic hydrochloric acid to replace water, then with ether, and dried in vacuo over solid sodium hydrate. Yield—about 7 gm. Arsenic content—32.0 per cent, practically insoluble in methyl alcohol, gives a very viscous solution with 16 parts of water



at room temperature. On heating for a short time in the water-bath, this solution becomes thin, and remains so after cooling.

*Preparation No. 3.*—The remaining 14 gm. of base was dissolved in caustic soda and converted into the dihydrochloride essentially as described by Kober. Yield—about 6 gm. of dihydrochloride. Arsenic content—31.77 per cent, practically insoluble in methyl alcohol, solution in 16 parts of water at room temperature very viscous, becoming thin when heated and then remaining thin and limpid on cooling.

*Reduction II. Simultaneous reduction by means of hypophosphorous acid and potassium iodide.*

*Preparation No. 4.*—This reduction was carried out as suggested in D. R. P. 271894. Since Fargher and Pyman note that they could not confirm the reduction of the nitro group, following this patent, the method will be described in more detail. Forty gm. of nitrophenol arsenic acid dissolved in 140 c.c. of glacial acetic acid, 100 c.c. water, and 100 c.c. of 50 per cent hypophosphorous acid. This was heated to 75° C. in about 20 minutes, with stirring, during which time a yellow precipitate of the arsenonitro compound separates. At this point a concentrated solution of 24 gm. of potassium iodide was added. A violent reaction occurred on the first addition and the precipitate increased, the temperature rising quickly to 105° C., heating being discontinued. The mixture was kept at about 100° C. with stirring, and after a total time of about 1½ hours, the solution became clear. It was then cooled to 20° C. and poured with stirring into 500 c.c. of concentrated hydrochloric acid at about 10° C. The precipitate was filtered, washed with ethyl alcoholic hydrochloric acid, then ether, and dried in vacuo. Yield—20 gm., pink in color, which deepens to brownish red, even after packing in evacuated ampules. Arsenic content—33.7 per cent. Required hot water to obtain a solution, forming gelatinous insoluble lumps with cold water. Insoluble in methyl alcohol.

The following reductions are of the progressive type, in which the nitrophenol arsenic acid was first reduced by means of ferrous hydroxide in alkaline medium, following directions given by Jacobs and Heidelberger<sup>9</sup>, and the amino phenol arsenic acid isolated.

*Reduction III. Reduction of the amino phenol arsenic acid by hypophosphorous acid and potassium iodide.*

Twenty-three gm. amino-phenol arsenic acid was mixed with 125 c.c. water and 125 c.c. of 50 per cent hypophosphorous acid. After adding 12 c.c. of 5 per cent potassium iodide solution, the mixture was heated for one hour to 60° C. in a carbon dioxide atmosphere. The clear solution gradually became very thick and viscous. It was cooled, diluted with 500 c.c. water, and divided into two equal parts.

*Preparation No. 5.*—One-half of the solution was made slightly alkaline with sodium carbonate, whereupon the arseno base was thrown out as a green voluminous slimy precipitate. This was filtered by suction and washed with water, then dissolved in 50 c.c. methyl alcohol and a sufficient quantity of hydrochloric acid in methyl alcohol solution. The addition of the hydrochloric acid produced at first a strong gelatinization, which thinned out again on the addition of more acid. The clear red solution was precipitated by ether in the usual way. Yield—6 gm. The product was obviously very impure due to decomposition of the base, and was rejected for purposes of comparison.

*Preparation No. 6.*—The other half of the reduction solution was precipitated by pouring into a mixture of 1,000 c.c. hydrochloric acid (1.19) and 400 c.c. water, cooled to 10° C. The finely shredded precipitate was filtered by suction, washed, and dried in vacuo over caustic. Yield—6.5 gm. Arsenic content—31.7 per cent, insoluble in methyl alcohol, very viscous solution in water, 1-16. The aqueous solution behaved in the usual way, becoming thin on heating, and remaining so after cooling.

*Reduction IV.*

Twenty-three and four-tenths gm. aminophenol arsenic acid was dissolved in a mixture of 150 c.c. water, and 50 c.c. hydrochloric acid. 2 gms. potassium iodide were added and the solution saturated with sulphur dioxide. After standing one hour, air was blown

through the solution until no odor of sulphur dioxide could be noticed. It was then neutralized with ammonia and filtered. Sodium hydrosulphite was then added to the filtrate, and the mixture warmed to 60° C. for about one hour, until a test showed the reduction to be complete. The fine yellow base was filtered, and washed thoroughly. The yield of moist base was 32 gm.

*Preparation No. 7.*—One-half of this was dissolved in methyl alcoholic hydrochloric acid and precipitated by ether. The dry hydrochloride weighed 8 gm. Arsenic content—31.7 per cent. Characteristics are those of the usual product from nitro phenol arsinic acid by hydrosulphite reduction. Soluble in water of room temperature without apparent viscosity.

*Preparation No. 8.*—The other half of this lot of base was dissolved in sodium hydroxide and precipitated by hydrochloric acid in the usual way. Yield about 7 gm. Arsenic content—31.5 per cent, insoluble in methyl alcohol, viscous solution with water.

*Reduction V. Reduction of amino acid to arsenoxide and completion by means of hypophosphorous acid.*

Twenty-three gm. amino phenol arsinic acid dissolved in 150 c.c. water, 50 c.c. hydrochloric acid added and then a solution of 2 gm. potassium iodide in 20 c.c. water. The clear solution was saturated with sulphur dioxide and after one hour, was freed from sulphur dioxide by blowing air through, and filtered. To this solution, 100 c.c. of 50 per cent hypophosphorous acid was added and the mixture allowed to stand 2 hours.

*Preparation No. 9.*—One-half of reduction mixture was diluted to 450 c.c. and made slightly alkaline with sodium carbonate. The base was precipitated in yellow flocks which rapidly darkened and finally became dark green. After filtering and washing, it was dissolved in methyl alcoholic hydrochloric acid and precipitated by ether. The dihydrochloride was normal in appearance. Yield—6 gm. Arsenic content—32.1 per cent, almost clear, soluble in 10 parts of methyl alcohol. It dissolved in 16 parts of water forming a solution which showed a slightly viscous behavior, but not nearly so marked as in preparation No. 10.

*Preparation No. 10.*—The other half of the above reduction mixture was run into 500 c.c. 1-1 hydrochloric acid, the precipitate filtered off, washed and dried in the usual way. Yield—7.5 gm. Arsenic content—31.5 per cent, insoluble in methyl alcohol. Very viscous solution with water 1-16.

*Reduction VI. Reduction of the amino phenol arsinic acid by sodium hydrosulphite.*

Twenty-three gm. of amino acid were dissolved in 120 c.c. water and 60 c.c. caustic soda 2 N. The solution was added to a solution of 250 gm. of sodium hydrosulphite in 1200 c.c. water, and the mixture warmed to 60° C. until reduction was complete (1½ hour). The yellow base was filtered, washed and divided into 2 parts.

*Preparation No. 11.*—One half was dissolved in methyl alcoholic hydrochloric acid in the usual way and precipitated from ether. Yield—8 gm. Arsenic content—29.8 per cent. Soluble with slight turbidity in methyl alcohol, soluble in cold water giving a very slightly viscous solution.

*Preparation No. 12.*—The other half of this base was dissolved in caustic soda, acidified with hydrochloric acid, and precipitated from 1-1 hydrochloric acid at low temperature. The product stuck badly to the filter paper and could not be recovered in a clean state. It was insoluble in methyl alcohol and formed a very gelatinous solution with cold water.

*Reduction VII.*

The next product was made following the procedure described by Kahn<sup>13</sup>. Attempts to prepare amino hydroxy phenyl arsine by the complete reduction of nitro phenol arsinic acid did not give satisfactory results. It was found, however, that good yields of arsine could be obtained by the reduction of arspenamine.

*Reduction of arspenamine to the primary arsine.*

Thirty grams of arspenamine were dissolved in 300 c.c. of water and 75 c.c. hydrochloric acid. The solution was stirred and 50 gm. of zinc dust added gradually, adding 100 c.c. more hydrochloric acid in portions to keep up the evolution of hydrogen, keeping the temperature below 30° C. all the time. When the action was over, 300 c.c. water were added,

and the solution warmed on the water-bath, adding more acid and zinc dust until the solution became practically colorless. This was then cooled, sodium acetate in excess added, and extracted with ether. The ether extract was dried with sodium carbonate and evaporated, leaving a residue of 24 gm. of white crystalline solid whose characteristics corresponded to those of the primary arsine.

Eighteen and five-tenths gm. of this arsine were dissolved in 200 c.c. of ethyl alcohol and 100 c.c. 2 N hydrochloric acid. The clear solution was added to a solution of arsenoxide prepared as follows: 23 gm. amino phenol arsinic acid were dissolved in 200 c.c. water and 100 c.c. hydrochloric acid 2 N 6 gm. potassium iodide were added, the solution saturated with sulphur dioxide and allowed to stand 2 hours. After mixing, the solution was allowed to stand for 2 hours, and then divided into 2 equal parts.

*Preparation No. 13.*—One half of this solution was neutralized with sodium carbonate, the base filtered off, dissolved in methyl alcoholic hydrochloric acid and precipitated from ether. Yield—10 gm. Arsenic content—31.7 per cent, readily soluble in methyl alcohol, and in cold water, giving a thin, nonviscous solution.

*Preparation No. 14.*—The other half of the above solution was poured with stirring into 1000 c.c. of 1-1 hydrochloric acid at 0° C., the precipitate filtered, washed and dried. Yield—12 gm. Arsenic content—31.96 per cent, completely insoluble in methyl alcohol, and forms a thick gelatinous solution with 16-20 parts of water.

*Preparation No. 15.*—Nine gm. of arspenamine, prepared as in experiment No. 1, were dissolved in 90 c.c. water. The resulting thin limpid solution was precipitated by pouring into 900 c.c. 1-1 hydrochloric acid at 10° C. The product was filtered, washed and dried in the usual manner. Yield—7 gm. Arsenic content—32.2 per cent insoluble in methyl alcohol, difficultly soluble in water giving a thick viscous jelly.

*Preparation No. 16.*—Six gm. of arspenamine, prepared as in experiment No. 14, were dissolved in 400 c.c. hot water. After cooling, the solution was made alkaline with sodium carbonate, the base filtered off, washed, dissolved in methyl alcoholic hydrochloric acid and precipitated from ether. Yield—3 gm. Arsenic content—31.77 per cent, clear solution with methyl alcohol, soluble in 16 parts of cold water without noticeable viscosity.

*Preparation No. 17.*—Six gm. of arspenamine, prepared as in experiment No. 3, were precipitated as above from ether, yielding about 4 gm. of a product which gave a slightly turbid solution in methyl alcohol, and showed only a slight viscosity when dissolved in 16-20 parts of cold water. Arsenic content—31.75 per cent.

#### SUMMARY

A number of different arspenamines have been prepared by varying the process of reduction and precipitation. The method of reduction produces certain differences in the characteristics of the arseno base, in regard to stability and extraneous impurities. On conversion into the dihydrochloride, precipitation by means of ionized solutions yields in every case products whose gelatinous characteristics, as evidenced by the viscosity of aqueous solutions and insolubility in methyl alcohol, are much more marked than when the dihydrochloride is prepared by the use of anhydrous non-electrolytes. This may be explained by the fact that arspenamine is inherently a colloidal substance, and that electrolytes in certain concentrations produce coagulation of the disperse phase of its emulsoid sols. These macroscopic observations on the colloidal variability of arspenamine, suggest the occurrence of less obvious, but similar differences in the disperse state of solutions prepared for intravenous injections, and consequently have a direct bearing on biological and clinical results obtained by their use.

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### III. THE VALUE OF THE REFRACTO-VISCOSIMETRIC PROPERTIES OF THE BLOOD SERUM IN CASES OF TUBERCULOSIS\*

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THE viscosity and refractivity of blood serum and their intimate relation have been studied in syphilis and cancer.<sup>2, 3</sup> I further found a deviation from the normal in occasional cases of actinomycosis, blastomycosis, sarcoma, and tuberculosis. In order to observe such deviation in patients with tuberculosis, I made a special study of 160 patients at the Glenn Lake Sanatorium. The condition of the patients was carefully analyzed and classified by the staff of the sanatorium. The technic used in making this study was essentially the same as that applied in the previous studies.<sup>2, 3</sup>

The cases were divided for study, according to the stage of the disease, into four groups: (1) Arrested cases in which there had been no signs of active tuberculosis for at least three months, and the patients were in good condition; (2) stationary cases in which neither progress nor improvement was taking place; some of the patients in this group were apparently in good condition; others were in an advanced stage of the disease; (3) cases in which the objective signs of tuberculosis were diminishing, and the patients "improving"; this group comprised patients who were still very sick, and others whose symptoms might soon be arrested; and (4) cases in which the signs of activity were of a progressive type. The four groups are divided in the chart by horizontal lines and subdivided by vertical lines.

Viscosity is expressed in relation to the viscosity of distilled water. In this series the large model of the viscosimeter of Hess was used, whereby a constant temperature of 20° C. was maintained.

Refractivity is expressed in the units of Pulfrich.<sup>3</sup>

Globulin percentage was obtained by interpolation with the aid of Naegeli's chart.<sup>3</sup> This percentage claims only relative value for reasons explained in previous papers.

The refractoviscosimetric quotient attributes to each serum a certain index which is obtained through division of the refractive index (multiplied by ten) by the viscosimetric index. It illustrates the fact that serum, respectively, its colloidal component with a certain refractive power may vary in viscosity. Two serums, for example, were obtained with a refractive index of 60.0 (Pulfrich units). One showed a viscosity of 2.00, the other of 1.80. The refractoviscosimetric quotient is 300 in the first sample and 333 in the second; the higher the quotient the lower the viscous power of a certain colloid substance.

\*Received for publication, March 18, 1922.

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The normal range in each condition is represented by a shaded field in the chart. This range is based on a combination of my findings and those mentioned in the literature.<sup>5, 6</sup> It should be stated especially that the upper and lower limits are not absolutely sharp. The height of the black columns indicates the number of cases.

## DISCUSSION

The viscosity is normal in arrested cases in Group 1. Although the other three groups comprise cases in which the viscosity is normal, there is a marked preponderance of cases in which the viscosity is high. In the pro-

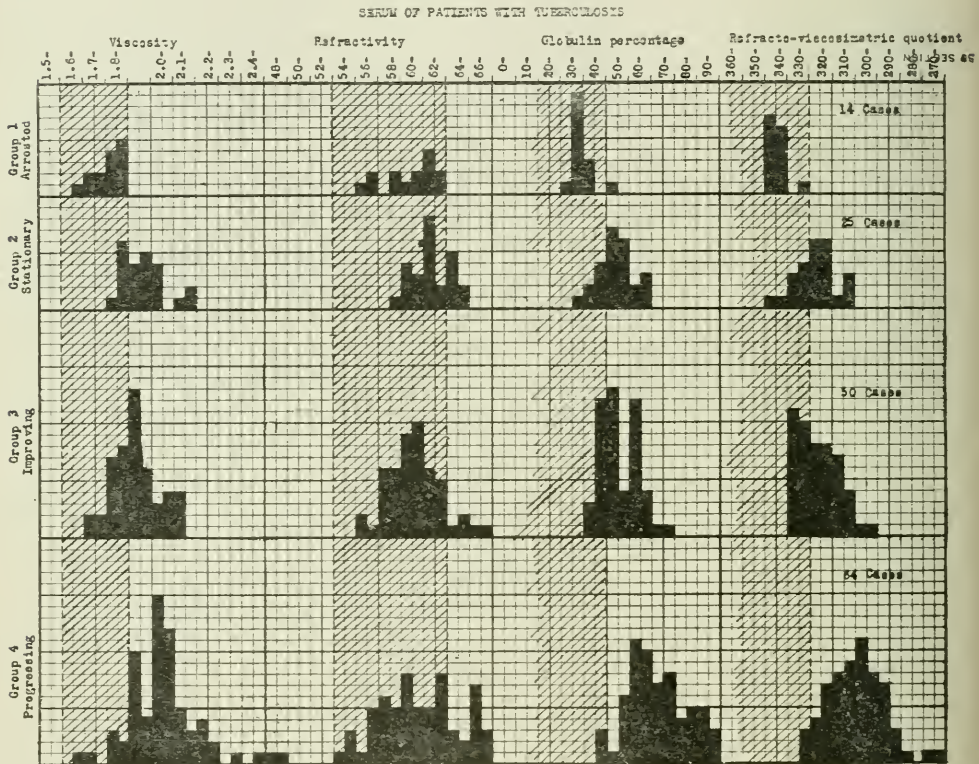


Fig. 1.

gressive stage of the disease (Group 4) the viscosity is greatly increased. Almost one-half of all the serums of this group have a viscosity between 2.00 and 2.10. A viscosity of 2.30, 2.40, and even of 2.60, as found in several instances, is to be considered as exceptionally high. Such degrees of viscosity were not observed in the great number of determinations made during the past year in other diseases. It is also worthy of note that in the few cases of Group 4 in which the viscosity was normal, the refractivity was extremely low. A viscosity of from 1.40 to 1.60 under usual conditions would correspond to such low refractive indexes. If, therefore, a viscosity of from 1.70 to 1.90 is found, this "normal" viscosity is very much elevated.

The refractivity of tuberculous serum is normal in most cases. The field

between 54 and 64 (Pulfrich units) corresponds, according to Reiss, to serums containing from 7 to 9 per cent of dissolved protein. Between these two percentages, Reiss declares, every normal serum may be ranged. It is, therefore, probable that the total amount of protein in the serum of tuberculous patients is not increased, or only slightly. This would indicate that the protein has a greater viscosimetric effect, or that it is not of a normal composition. The change that occurs in the protein is not explained by these tests. On the basis of the experiments of Naegeli and his collaborators it may be a change in the ratio of albumin and globulin. It is known that the viscosity responds very easily to a change in this ratio, while the refractivity is but little influenced by it.

The albumin-globulin ratio, as obtained with the aid of Naegeli's chart is reproduced in the third column, whereby only the globulin percentage is shown. As expected, the globulin is high, especially in cases of progressive tuberculosis.

The refractoviscosimetric quotient gives a very similar picture. It indicates that in tuberculosis the viscosity of the serum is abnormally high in relation to the refractivity. The refractoviscosimetric quotient classifies a number of cases as abnormal which in the first column showed normal viscosity. This is especially true in cases of Group 4. There are some exceptions. Three of the sixty-four patients with progressive tuberculosis showed normal refractoviscosimetric quotient. It was not possible to find a satisfactory explanation for this. One patient, however, had lost all resistance to the infection. On the other hand, the lowest values belong to patients in whom the disease is rapidly progressing and fatal. In Groups 2 and 3 half of the patients had normal rates. According to our classification these patients are in better condition than those with lowered rates. It did not seem advisable to subdivide the groups further on account of the limited number of cases.

Of the 160 patients examined the findings of 153 are discussed. The other seven patients did not have tuberculosis, but it should be noted that a decreased refractoviscosimetric quotient was found in those affected with blastomycosis, severe bronchiectasis with abscess, maxillary and other sinuses, recent catarrhal infection, and lead poisoning. In one case of peritonitis the refractoviscosimetric quotient was normal, in another it was definitely low. In tuberculosis the refractoviscosimetric quotient of the serum has a definite relation to the stage of the disease. The severity of the disease finds its expression in an accordingly low rate.

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# THE ACTIVATION OF AN ENZYME POISONED BY HEAVY METAL SALTS\*

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## I. INTRODUCTION

IT HAS been shown, previously, that the coagulation of gelatin, through the action of various heavy metal salts, is reversible<sup>1</sup>; that by means of properly chosen salts of the alkali and alkaline-earth metals, the coagulated protein may be brought back to its original state. Since the toxic effects of heavy metals upon protoplasm is generally considered to be due to the coagulation of the proteins, and since the coagulated proteins may be restored to their normal physical state, it seemed reasonable to think that this end should be sought in a rational therapy devoted to combating the effects of heavy metal poisons. However, the explanation of the beneficial effects of the administration of neutral and alkaline salts in heavy metal poisoning can be attributed to this action of these salts, only insofar as the restoration of protoplasm to its normal physical state includes the resumption of its physiologic functions. The activity of the enzymes is so characteristic of normal protoplasmic behavior, that the idea suggested itself to me that like protein some typical enzymatic reaction mixture ought also to be capable of being "poisoned" by heavy metals and subsequently capable of "cure," through the addition of properly chosen lighter metals.

*The following experiments, carried out upon saliva, show that this secretion behaves like the inert protein of our previous experiments, in that properly chosen neutral salts of the alkali and alkaline-earth metals restore it to its normal appearance and activity, after its coagulation and complete inactivation, through the action of heavy metal salts.*

## II. EXPERIMENTS

Fresh saliva was obtained by chewing paraffin, the same sample being employed in each series of related observations. A two per cent starch paste, made in the usual way from a commercial corn starch, was used as the substrate. The activity of the enzyme on the starch was tested qualitatively with Fehling's solution, after incubation of the substrate-enzyme mixture in a water bath at 40° C. Quantitative estimations were made with Benedict's quantitative solution, the amount of residual blue color being used as an index to the relative quantities of reducing sugars present.

1. *Inactivation of Saliva.*—When mercuric chloride is added to saliva up to a final concentration of  $1/200$  M. a heavy flocculent precipitate is obtained,

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Received for publication, March 20, 1922.



surmounted by a somewhat opalescent fluid. Lower concentrations of mercuric chloride produce less precipitate which also settles out more slowly. Higher concentrations of mercuric chloride produce a more rapid and complete settling out of the coagulum, so that the supernatant fluid becomes almost clear. In the lower concentrations of mercuric chloride the starch splitting ferments are inhibited. In any concentration of mercuric chloride above  $\frac{1}{200}$  M. they are completely inactivated. Complete coagulation and inactivation requires from half an hour to an hour at room temperature, at the end of which time the heavy metal is firmly bound to the material composing the precipitate, as proved by the fact that the precipitate obtained

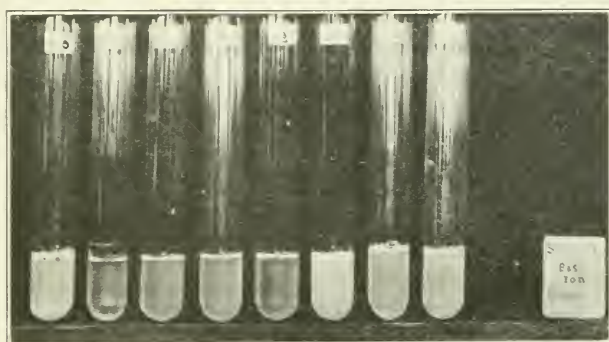


Fig. 1.

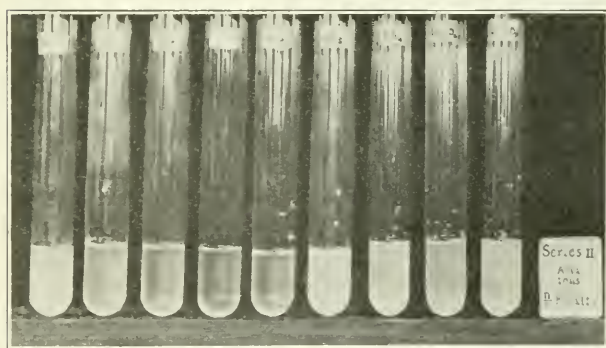


Fig. 2.

from 150 c.c. of saliva with mercuric chloride at a final concentration of  $\frac{1}{200}$  M. in saliva, when filtered off and washed repeatedly with distilled water, failed to darken a bright copper wire placed in the dialysate obtained from the precipitate. Mercury could not be detected in the water surrounding the capsule though dialysis was continued for twenty days.

2. *Resolution of Mercurialized Saliva.*—(1) At various times (one hour to three months) after mercurial inactivation of saliva, small quantities of it were treated with various concentrations of neutral and alkaline salts of the alkali and alkaline-earth metals (after thorough shaking so as to obtain a homogeneous suspension). When sufficiently high concentrations of the chlo-

rides, bromides, iodides and sulphocyanates of ammonium, potassium, sodium, strontium, barium, calcium and magnesium were employed (omitting those not sufficiently soluble) most of the precipitate "dissolved" and the mixture again became a homogeneous opalescent liquid. The nitrates, sulphates, citrates, acetates and carbonates had little or no ability to produce these changes. The salts of strontium, barium and calcium caused a secondary precipitation of a white inorganic salt (sulphate?) along with the resolution of the original precipitate. Simultaneously with the resolution of the precipitate the starch splitting activity of the mixtures was restored. Those salts (nitrates, sulphates, citrates, acetates and carbonates) which did not resolve the precipitate, did not reactivate the enzyme. While resolution of the precipitate and reactivation of the ferment began quickly, restoration to normal appearance and behavior did not occur until several hours had passed.

(2) The speed and degree of the restoration of the ferment were affected by the concentration of the salt employed as the inactivator, and by the concentration of the salt employed as the reactivator. At a concentration of  $\frac{1}{200}$  M. mercuric chloride in the mixture, the addition  $\frac{1}{10}$  N. salt of the alkali or alkaline-earth metals produced little or no effect. Normal concentrations of such salts, when added to the inactivated saliva, produced the marked effects seen in Figs. 1 and 2. However, when the concentration of mercuric chloride was raised to  $\frac{1}{50}$  N. or more, normal concentrations of the lighter salts had only a slight effect in resolving the precipitate, and no effect in activating the enzyme.

(3) The effects obtained by the use of the salts of the alkali and alkaline-earth metals were not due to a precipitation of the heavy metal in insoluble form, but to a displacement of the heavy metal from its combination with the material in the saliva, leaving the heavy metal in a soluble, diffusible state. This was shown by a heavy coating of mercury deposited upon a bright copper wire placed in the liquid surrounding the dialyzing capsule and contents

TABLE I

EFFECT OF BASIC RADICALS IN A SERIES OF CHLORIDES  
STARCH SPLITTING ACTIVITY OF MERCURIALIZED SALIVA AFTER THE ADDITION OF A SERIES OF CHLORIDES

	TUBE CONTENTS	APPEARANCE AFTER 24 HOURS	COLOR OF ADDED BENEDICT'S SOLUTION
1	5 c.c. HgCl <sub>2</sub> -saliva mixture + 5 c.c. H <sub>2</sub> O	Turbid, opaque with precipitate unaffected	Original blue unchanged
2	5 c.c. " " " + 5 c.c. N NH <sub>4</sub> Cl	Homogeneous—almost clear	Yellowish green; almost complete reduction
3	5 c.c. " " " + 5 c.c. N KCl	Homogeneous—less transparent than (2)	Pale blue
4	5 c.c. " " " + 5 c.c. N NaCl	Homogeneous—less transparent than (3)	Blue—deeper than (3)
5	5 c.c. " " " + 5 c.c. N MgCl <sub>2</sub>	Practically same as (4)	Blue—very slightly deeper than (4)
6	5 c.c. " " " + 5 c.c. N CaCl <sub>2</sub>	Secondary white precipitate	Yellowish blue—deeper than (2)
7	5 c.c. " " " + 5 c.c. N BaCl <sub>2</sub>	Secondary white precipitate	Almost the same but slightly paler than (6)
8	5 c.c. " " " + 5 c.c. N SrCl <sub>2</sub>	Secondary white precipitate	Paler than (7)—deeper blue than (2)

described above, within a few minutes after the introduction of 10 c.c. of molar potassium bromide into the dialysis capsule.

(4) The salts which bring about restoration of the enzyme are not all equally effective. The following experiment shows this and serves to illustrate the methods of our quantitative experiments. Mercuric chloride was added to fresh saliva up to a final concentration of  $\frac{1}{200}$  M. in the mixture. Twenty-four hours later the mercurialized saliva was shaken thoroughly and introduced into test tubes in 5 c.c. amounts. The subsequent treatment of these tubes is shown in Tables I and II and Figs. 1 and 2. Twenty-four hours after the additions indicated, 0.5 c.c. of the contents of each tube was removed and mixed with 5 c.c. of two per cent starch paste, in small tubes of uniform thickness and diameter. After being carefully shaken in a rack and introduced simultaneously into a water bath at 40° C. for half an hour, they were cooled in cold tap water for a few minutes. One c.c. of the topmost (clear) fluid of each tube was quickly transferred to small tubes of the same thickness and diameter, and to each tube 5 c.c. of Benedict's quantitative sugar reagent were added from a burette. The tubes were shaken in a rack for a few seconds to insure admixture, immersed simultaneously in a boiling water bath, and after 20 minutes were subjected to a color comparison by means of the unaided eye, the residual blue color of the unchanged cupric salt being used as the reciprocal index to the relative quantities of the reduc-

TABLE II

EFFECT OF ACID RADICALS IN SERIES OF POTASSIUM SALTS  
STARCH SPLITTING ACTIVITY OF MERCURIALIZED SALIVA AFTER THE ADDITION OF A SERIES OF POTASSIUM SALTS

	TUBE CONTENTS	APPEARANCE AFTER 24 HOURS	COLOR OF ADDED BENEDICT'S SOLUTION
1	5 c.c. HgCl <sub>2</sub> -saliva mixture + 5 c.c. H <sub>2</sub> O	Turbid, opaque with precipitate unaffected	Original blue unchanged
2	5 c.c. " " " + 5 c.c. N KCl	Homogeneous, slightly opaque	Pale blue
3	5 c.c. " " " + 5 c.c. N KBr	Homogeneous, almost transparent	Yellow, practically complete reduction
4	5 c.c. " " " + 5 c.c. N KI	Homogeneous, practically same as (3)	Faint greenish yellow
5	5 c.c. " " " + 5 c.c. N KCNS	Homogeneous, slightly opaque	Blue, slightly deeper than (2)
6	5 c.c. " " " + 5 c.c. N KNO <sub>3</sub>	Same as (1)	Original blue unchanged
7	5 c.c. " " " + 5 c.c. N K <sub>2</sub> SO <sub>4</sub>	Same as (1)	Original blue unchanged
8	5 c.c. " " " + 5 c.c. N KC <sub>2</sub> H <sub>3</sub> O <sub>2</sub>	Slightly clearer than (1)	Original blue unchanged
9	5 c.c. " " " + 5 c.c. N KC <sub>6</sub> H <sub>5</sub> O <sub>7</sub>	Same as (1)	Original blue unchanged

ing sugars formed. (Only differences obvious to the unaided eye were considered significant.) The uniform results obtained from several repetitions of the experiment are recorded in the last column of Tables I and II. A tabulation of these results indicates the relative values of the various radicals, in the order of their potency in restoring the poisoned enzyme to be as follows, that most powerful being mentioned first:

*Basic Radicals*

Ammonium  
Strontium  
Calcium  
Potassium  
Sodium  
Magnesium

*Acid Radicals*

Bromide  
Iodide  
Chloride  
Sulphocyanate

(5) The enzyme coagulated and completely inactivated through the action of mercuric chloride may be restored to 80 per cent of its normal starch splitting activity. Equal quantities of fresh saliva and of mercurialized saliva reactivated after forty-eight hours with ammonium chloride were allowed to react upon starch paste as above, and color comparisons were made quantitatively by means of a Bock-Benedict colorimeter. Repeated experiments showed a uniform restoration of the previously inactivated enzyme to 80-85 per cent of the efficiency of the fresh saliva.

3. The foregoing experiments were repeated in part using silver nitrate as the inactivating agent. This salt coagulated and completely inactivated saliva at a final concentration of  $\frac{1}{50}$  N. in the saliva. In general, the results of the experiments were the same as those obtained in the case of mercuric chloride except that higher concentrations of the light metal salts were necessary to produce the effects.

The common lead salts proved to be incapable of completely inactivating the salivary ferment, so that experimentation with them was abandoned.\*

### III. DISCUSSION OF RESULTS

In explanation of the restoration of coagulated proteins to their normal physical state, through the action of light metal salts, we expressed the view that the process consists in the conversion of insoluble, unhydrated heavy metal protein compounds into more soluble hydrated light metal protein compounds;<sup>1</sup> that these compounds of metals and proteins may be considered analogous to soaps formed by the combination of heavy and light metal oxides and hydroxides with fatty acids; in that the heavy metal soaps are uniformly slightly soluble and slightly hydrated, while the light metal soaps are soluble and highly hydrated.<sup>2</sup> The extension of this conception to the explanation of our observations on saliva, necessitates the following conclusions:

(1) That the enzyme is a protein, or that it is intimately associated with a protein, which is soluble and active only when combined with certain salts or metals.

(2) That it is capable of combining with other inorganic salts, so as to form a series of metal-protein-acid compounds, analogous in general, in solubility and hydration capacity (though not necessarily exactly so) to the soaps formed by the neutralization of fatty acids with metallic hydroxides.

(3) That such heavy metal salts as inactivate the enzyme, do so through the formation of insoluble, dry, heavy metal-protein-acid compounds, thus removing the active catalyst from the solution.

(4) That such lighter metal salts as reactivate the inert enzyme do so

\*Incomplete experiments with pepsin indicate that this enzyme may be reactivated after complete inactivation through the action of mercuric chloride.



by replacing the heavy metal of the compound with the light metal of the introduced salt, forming a new compound—a soluble and hydrated and hence active light metal-protein-acid compound.

(5) That the effect of the acid radicals is due to their combination with the protein to form various compounds differing from each other in solubility and hydration capacity.

The facts obtained in our experiments and in those of others are in agreement with these conclusions. That the enzyme is not active except in the presence of various salts is shown by the observation of numerous investigators,<sup>3</sup> that dialysis completely inactivates the salivary enzyme, (a precipitation occurs in the dialysis tube) but the addition of any one of several neutral salts almost immediately restores the ferment to its activity, (this being accompanied by resolution of the precipitate obtained in dialysis). This may be interpreted as meaning that the active ferment is a metal-protein-acid compound, which upon hydrolysis yields an insoluble protein as a precipitate in the dialysis tube.

That stable compounds of the enzyme with heavy metals are formed when the saliva is treated with heavy metal salts, is strongly suggested by the fact that the precipitate so obtained does not release the metal after long dialysis.

That a new compound is formed, and the heavy metal freed from combination when the proper salt is added to the heavy metal enzyme precipitate, is indicated by the observation that such treatment causes an almost immediate restoration of the starch splitting activity of the enzyme, and a simultaneous diffusion of the hitherto undiffusible heavy metal through a dialysis tube.

It should be pointed out that the impurity of the enzyme (or enzymes) employed in the experiments must be taken into account in a comparison of quantitative results. The presence of salts other than those added experimentally, especially such as cause precipitation in the mixture, cannot be without effect. This factor alone may explain the apparent abnormal position of certain of the alkaline-earth metals in the table of effective salts. It is also probable that proteins present in saliva other than the active enzyme (presumably a protein) have definite effects on the behavior of the salts added and on the enzyme itself. The manifest difficulty of obtaining a pure ferment has up to the present time prevented our taking adequate account of these factors.

#### IV. PRACTICAL APPLICATIONS

Whether or not our explanation of our observations is correct, the facts without explanation are important not only for a better understanding of the nature and behavior of enzymes, but for the better therapy of heavy metal poisoning in living tissue. Our original question as to whether living material coagulated by heavy metals could be restored to function as well as to normal physical state through the action of salts of lighter metals may be considered answered. This brings us to restate a conclusion based upon earlier experimentation, that in the treatment of heavy metal poisoning, our efforts should be devoted chiefly to the restoration of the coagulated tissue

to its normal state and to maintaining it in this normal state till the injurious agent shall have been removed.

Our experiments indicate the probability that not only can tissue coagulated by heavy metals be restored to normal state and behavior but that the heavy metal can be hastened out of the body by maintaining it in soluble and diffusible form.

Neither is there a lack of clinical<sup>4</sup> and experimental<sup>5</sup> evidence that these results can be accomplished.

A restatement of the method to be employed in the treatment of individuals poisoned by heavy metals may not be amiss. In order to combat the primary coagulative effects of heavy metals upon the tissues it is necessary to obtain, at the earliest possible time, a high concentration of suitable salts in the body and to maintain this high concentration over a long period of time. The salts which can best be employed for this purpose are the salts of potassium and sodium. Because of the buffer action of the tissues of the body and because of the ready oxidation and elimination of such organic acids as citric, acetic and carbonic the objection to the use of salts of these acids, as would be concluded at first thought from the examination of the experimental data, will not obtain. On the contrary these are the ones which should be employed because they are easily borne at high concentrations in the body. In addition these salts will serve best to combat the secondary "acidosis" which is the constant accompaniment of heavy metal poisoning. The alkaline salts of sodium and potassium, then, should be administered in large doses and should be maintained in the body up to the limit of tolerance, till such a time as the heavy metal has been excreted from the body.

#### V. SUMMARY

1. The enzyme in saliva inactivated (coagulated) through the action of the heavy metal salts  $\text{HgCl}_2$  and  $\text{AgNO}_3$  may be reactivated by means of sufficiently high concentrations of certain neutral salts of the alkali and alkaline-earth metals.

2. The degree and rate of reactivation of the coagulated enzyme is affected by the concentration of the coagulant and the reactivating salts and by the basic and acid radical of the salt employed as the reactivator.

3. Facts are set forth, indicating the probable protein nature of the enzyme, together with the probability of the formation of various compounds of salts with this protein.

4. A restatement is made of the principles underlying a rational therapy of heavy metal poisoning.

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## THE EFFECT OF BILE SALTS IN THE URINE ON ROUTINE TESTS FOR ALBUMIN\*

BY SYMMES F. OLIVER,† M.D., CINCINNATI, OHIO

IN THE course of some investigations on bile, I became interested in the urinary findings of patients suffering from various diseases of the liver and the biliary passages.

My attention was early directed to the frequency with which albumin is reported in the urine of such patients, particularly those suffering from jaundice of hepatic origin. Often, albumin is reported without casts or other evidences of renal disease. In cases of common duct obstruction, urinalysis almost invariably showed albumin while text books discussing the question, speak of albuminuria as a frequent complication.

Routine urinalysis performed on a large number of cases of obstructive jaundice revealed the following: Practically all gave positive tests for albumin with the acetic acid and heat tests, Heller's cold nitric acid test and Robert's reagent. In a few cases a double ring, one just above the other, was obtained with  $\text{HNO}_3$  and with Robert's reagent. These rings formed immediately and became stronger on standing. The upper ring on standing tended to diffuse upward so that after about one hour, the whole upper layer of urine became clouded. The lower ring, exactly at the point of contact between the reagent and urine remained constant or became slightly accentuated. The occurrence of this double ring suggested that two different substances were present in the urine, which reacted with the reagents used. The lower ring exactly at the point of contact of the reagent and urine was found to be albumin.\* The upper ring was not due to urates since this ring only forms on standing for some little time, and tends to appear higher up in the urine than the ring under discussion. Likewise, urates do not interfere with the acetic acid test whereas the substance in question caused a decided clouding without the application of heat. Further, the urate ring clears up when heat is applied while the ring here described is not affected. Mucin and various nucleo-albumins may, under certain conditions, give reactions that lead to confusion. These substances may be ruled out, however, by testing for protein compounds and, if present, removing them from solution. Mucin causes a slight clouding with acetic acid and occasionally is present in sufficient concentration to produce a ring with nitric acid—or Roberts reagent. This ring, however, is a fine delicate crystalline ring which easily might escape detection. Mucin rings may be obtained for comparison by using nasal secretion where mucin exists in almost pure form. If mucin is suspected, dilution of the urine together with filtration would eliminate it as a factor. The ring described in this paper is a broad milky one just above the layer of contact between the

\*Received for publication, March 25, 1922.

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reagent and urine and tends to diffuse rather rapidly upward, ultimately clouding the entire urinary layer. In all cases where such reactions are obtained it would be advisable not to too readily assume that mucin and nucleo-albumins are the responsible agents.

Since the urines examined were all obtained from cholemic patients, it seemed logical to suppose that certain constituents of the bile might be the factors responsible for these changes. It is known that bile salts are precipitated from solution when treated with mineral acids and dilute acetic acid and that they can be redissolved on the addition of alkali. The usual reagents used in the common tests for albumin, such as nitric acid, hydrochloric acid and weak acetic acid, all bring about this change. Solutions of bile salts were made and tested with the above reagents. In all cases a ring such as described above, formed just above the layer of contact of the reagent and solution used. It was found that bile salts in dilutions as high as  $\frac{1}{64}$  of 1 per cent gave positive ring, and acetic acid tests. Small quantities of Sodium glycocholate and taurocholate were then added to normal urine and tests identical to the above were obtained.

Urines that were known to contain albumin were then treated with traces of bile salts and tested. Double rings such as previously described were obtained in all specimens examined.

The urines examined were then in all cases tested for bile salts. Four tests were used for each specimen, namely: Hay's sulphur test, the peptone test, the precipitation of egg albumen and the starch reaction. The latter two tests consisted in the use of 2 per cent egg albumen and starch solutions, weakly acidified with acetic acid and preserved with small amounts of salicylic acid. These reagents are rendered milky or definite precipitation occurs in the presence of bile salts. In the latter three tests, the urine was diluted so that the specific gravity was reduced to 1008, filtered if necessary and rendered faintly acid with acetic acid. Four c.c. of the reagents were used and the urine added drop by drop until a milkiness of the solution resulted. In all cases where these tests were made, a decided increase in bile salt content was found in the urine. In this way a check on all urines was obtained so that other ingredients could be excluded. In all cases of obstructive jaundice and the majority of cases of cirrhosis of the liver, a bile salt increase was found in the urine. It is noteworthy that in certain cases of cirrhosis of the liver, a decided increase in bile salt was found without any increase in bile pigment. Conversely, certain of these cases showed an increase in bile pigment with little or no augmentation of bile salts.

In all cases where an increase in bile salts was noted in the urine, it was observed that the urine was cloudy and turbid, if the reaction of the mixture was acid. This turbidity was increased if acid were added to the mixture. Neutral or alkaline urines were found to be almost always clear.

Roughly, this turbidity of acid urines was proportional to the bile salt content present. After cholecystostomy or cholecystectomy the urines usually cleared up within four to six days after operation. The urine becomes lighter colored, the bile salts decrease in amount and the turbidity no



longer occurs in acid urines. A study was made in this connection on the urinary findings before and after operations on the gall bladder, and the findings will be reported later. It may be briefly stated that the bile salt content of the urine was found to be dependent on the secretion of bile by the liver and the concentration of bile salts in the bile. In cases of hepatic insufficiency it was found that an increase in bile salts occurred in the urine, whereas in cases where the functional activity of the liver soon regained its normal balance, the bile salts likewise tended to disappear from the urine and appear in normal amounts in the bile. These findings, however, will be reported in detail in a subsequent communication.

In conclusion, the clinical importance of these findings is quite obvious. It is of decided importance to the clinician to know whether he is dealing with an ailment which is hepatic in origin or whether the pathology is indicative of renal disease. Two cases, illustrative of this point, may be mentioned. One case, seen by Doctor Robert Kehoe, of the Department of Physiology, was a young woman who was six months pregnant. Her urine had been examined and albumin was reported positive. She had a slight elevation of temperature, together with digestive disturbances and evidences of toxemia. It was feared that she was developing eclampsia and the question as to the advisability of emptying the uterus came up. Doctor Kehoe noticed that she had a slight jaundice, and on examining the urine, found that no albumin was present but that there was a decided increase in bile salts. As a result of these findings, no operative interference was resorted to and the woman was able to save her baby.

The second case was a patient in our own practice: A woman had been operated on for gall stones, but, after the operation, her convalescence was extremely slow and tedious. She complained of excessive fatigue, prostration and absolute loss of appetite. The sight of food nauseated her and she was unable to retain her nourishment. Urinalysis revealed a urine of high specific gravity, with moderate amounts of albumin and a few casts. A decided increase in bile salts was also noted. At this time, however, I was unaware of the effects of bile salts on the albumin tests and the patient was consequently treated for nephritis. She received five intravenous injections of Fischer's solution with no improvement other than an increased excretion of urine. Clinically she was absolutely not benefited by her treatment and, after a lingering illness in which she became progressively weaker and more emaciated, she ultimately died. The use of alkaline therapy in cases of cholemia I believe is certainly not indicated and this case was probably actually harmed by the treatment she received.

Other cases might be mentioned, but these two suffice to show the practical importance of bearing these points in mind.

In conclusion, I wish to express my deep appreciation to Doctor Martin H. Fischer, of the Department of Physiology, for the opportunity to carry on this work. I also wish to thank Doctor F. B. Samson, of this city, for his helpful counsel and kindly advice, and Doctor A. P. Matthews and Doctor Shiro Tashiro of the Department of Physiological Chemistry, for their valued aid.

## THE NATURE AND TREATMENT OF MUSCULAR ATROPHY\*

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### STATEMENT OF THE PROBLEM

THE invariable wasting of muscles which follows section of the motor nerve, or destruction of the anterior horn cells of the lower motor neurones supplying the muscle, has led to the theory that the nerve cell exerts a *trophic* influence on the muscle. Neither physiological experiments nor anatomical evidence has succeeded in demonstrating the nature of this trophic influence. By some writers, the influence is supposed to reside in special trophic fibers; by others, a peculiar kind of nerve impulse is assumed in order to explain the facts. Numerous observations have been made in which a trophic influence of the nervous system upon other tissues and organs than muscle, seemed to be demonstrated. The trophic ulcers of the cornea following injuries to the fifth nerve, and lesions of the bone such as Charcot's joint, are cited in this connection. Gradually however, evidence has accumulated to show that most of these phenomena can be explained in a more satisfactory manner upon other grounds. Again, it has been thought by some on the basis of Boeck's work<sup>2</sup> on the motor end plate, that there is a continuity of substance from the nerve fiber through the end plate to the muscle. Since the protoplasm is continuous from nerve to muscle the atrophy of the nerve is necessarily followed by the wasting of the muscle. Against this view, it may be urged that muscle and nerve cells react differently to vital stains and to drugs, so much so in fact, that the two tissues differ fundamentally from a physiological point of view. Furthermore, if more proof is needed, it is a well established fact that the denervated muscle retains its irritability to a faradic current long after such irritability is lost in the nerve.

There remains to be considered the time honored theory that the muscular atrophy following nerve injuries is due to disuse. In support of this view there is the undoubted fact that a muscle which is not used decreases in size and strength as is shown in man by the shrinkage of limbs paralyzed by an upper motor neurone lesion. The atrophy however, of a denervated muscle is much more rapid. Indeed, as the curve (Fig. 1) shown in this paper proves, agreeing as it does so closely with that of Langley, there must be some general law of physiology at work. A suggestion as to the nature of this law will be made later in this paper.

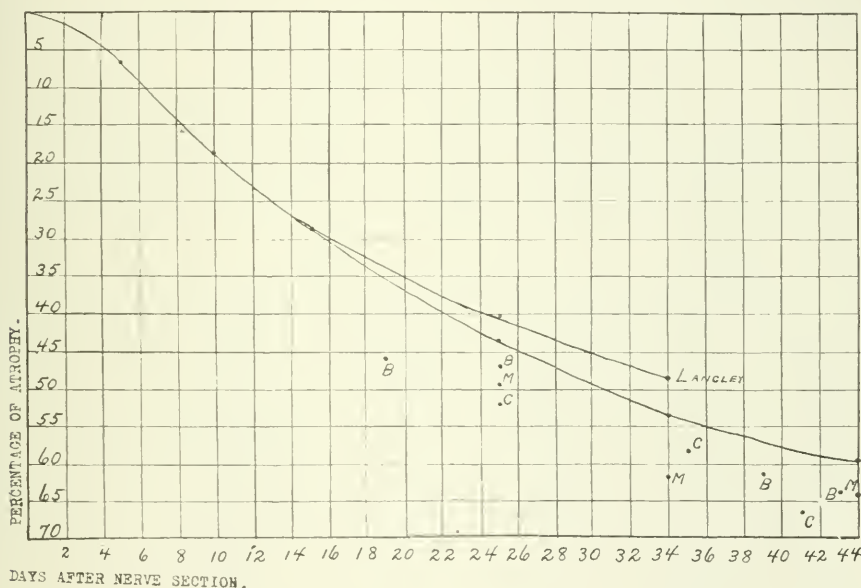
A practical consequence of great importance, of the disuse theory of atrophy is the treatment of atrophying muscle by electrical stimulation. Since 1841 following the lead of John Reid<sup>13</sup> it has been customary to treat mus-

\*Received for publication, April 11, 1922.

cular atrophy by causing the implicated muscles to contract by electricity, on the assumption that if this treatment did not prevent atrophy entirely, it would tend to lessen it. This traditional clinical opinion has again lately been voiced by Roberts.<sup>14</sup> Langley has reported a slight increase in size after long continued stimulation. Hartman and Blatz<sup>17</sup> in a recent paper were unable to demonstrate any beneficial effect from either galvanic stimulation or massage. At any rate, the influence of the electric current is probably not due to the production of contractions in the treated muscle as Cumberbatch<sup>3</sup> reports favorable results from the use of alternating sinusoidal currents which do not cause contractions.

A change of attitude towards the nature of muscular atrophy has recently come about through the work of J. N. Langley and his pupils. Langley and Kato<sup>7</sup> in the course of some experiments on the physiological action of

FIG. I



physostigmine had occasion to cut the sciatic nerve of rabbits. They noticed on the fifth day after nerve section that the gastrocnemius muscle was in a state of rapid, fibrillary contraction. These contractions are very small and can be seen best when the light falls at the proper angle on the wet, glistening surface of the muscle. This state of incessant activity persists throughout the whole period of muscular atrophy. This fact although recently confirmed by Langley was first described by Schiff<sup>15</sup> in 1851. In the course of experiments on the paralysis of the tongue in dogs following section of the hypoglossus on one side. Schiff observed the same fibrillary contractions in the atrophying muscles of the tongue. Schiff's language is so accurate and vivid that there can be no doubt that he was observing the identical fact described by Langley. Stevens<sup>16</sup> has confirmed and extended Schiff's experiment on the hypoglossus of dogs. The additional fact was brought out

by Stevens that the fibrillary contractions begin within three days after nerve section and persist throughout the whole period of atrophy, only to disappear with the regeneration of the nerve. There is therefore, an exact parallelism between the onset and duration of the atrophy and the appearance and continuation of the contractions. Beyond describing the fact, Schiff made no inference as to its significance. Langley however, impressed by the exact concomitance between the incessant fibrillar activity and the atrophy of the muscle has suggested that the former event is the cause of the latter. The wasting of a denervated muscle is really a fatigue effect caused by the ceaseless fibrillar activity. In support of this view of the facts Langley<sup>4</sup> and Barcroft and Kato<sup>1</sup> have shown that the specific gravity of atrophied muscle and fatigued muscle are less than that of normal muscle. Furthermore, as demonstrated by Langley and Itakagi<sup>6</sup> there is an increased use of oxygen in atrophied muscle in comparison with normal muscle, which they interpret to support the theory of fatigue by indicating an increased rate of breakdown.

#### OUR OWN EXPERIMENTS

The purpose of the experiments described in this paper is to test the theory that muscular atrophy is caused by incessant fibrillary activity. The particular method of attack here employed was suggested by certain experiments of Jacques Loeb<sup>10</sup> on the effect of certain ions on the rhythmical contractions of muscles. In the course of an investigation on the physiological activity of sodium and calcium ions, Loeb observed that skeletal muscles could be caused to contract rhythmically in a solution containing Na-ions. He used both NaCl and NaBr. The addition of a small quantity of Ca-ions or K-ions or both, inhibited these contractions. He had proved<sup>9</sup> earlier that Ca-ions decreased the excitability of skeletal muscles. Later<sup>11</sup> he established the fact that rhythmical contractions can be excited in smooth muscle by the addition of sodium ions and inhibited by calcium ions. He also showed<sup>12</sup> that a smaller amount of K- or Ca-ions are required to inhibit contractions in smooth muscle which contains no ganglia than in muscle which contains ganglia. The object of our experiments was to determine whether we could inhibit the fibrillar activity of denervated, skeletal muscle by the injection of certain salts of the Ca-group, and therefore prevent muscular atrophy. Langley has shown that the intravenous injection of calcium salts checked the fibrillar contractions. His experiment was not extended over a sufficient length of time to demonstrate any effect upon muscular atrophy.

The animals used in our experiments were guinea pigs. In each case, the right sciatic nerve was severed in the gluteal region and 6 mm. of nerve excised. Since accidental differences in the size of right and left groups of muscles are distributed according to chance, the possibility of a systematic error on this account is avoided by selecting the same leg for operation in all experiments. The possible influence of sex was left out of account. The wound healed always by first intention, the operation being usually bloodless. The animals were operated in groups of eight. Three pairs were then injected hypodermically with calcium, barium or magnesium salts, beginning the day



after the operation. It was found by experiment that the strongest concentration of salts which could be used without causing sloughing of the skin were  $\text{CaCl}_2\text{m}/4$ ,  $\text{BaCl}_2\text{m}/10$  and  $\text{MgSO}_4\text{m}/1$ . One cubic centimeter of the sterile aqueous solution of these salts was injected daily in the appropriate animal. Six guinea pigs were injected for 25 consecutive days; six for thirty-five days; and six for forty-four days, with some exceptions to be noted later. In each group of eight operated pigs there were two which served as controls by receiving no injection whatever. At the end of the time chosen for the test, the animals were killed and the gastrocnemius muscle on each side weighed. Each muscle was dissected out singly by the same person, in order to secure uniformity of dissection, and weighed as rapidly as possible on a sensitive balance, to avoid loss of weight from evaporation. The results of the experiments are shown in table I, in which the number of each animal in the series is given, together with the duration of the treatment, the salt used, the weight in grams of right and left gastrocnemius muscles, the difference in weights and the percentage of atrophy. The percentage of atrophy was calculated by dividing the weight of the left muscle into the corresponding difference of weight between right and left muscles.

The results of the experiments are also shown in graphic form in Fig. 1. The first part of the curve of atrophy is copied from Langley and Kato,<sup>8</sup> the latter part is drawn from our control pigs. The curve agrees with the con-

TABLE I

NO. PIG	DAY KILLED	INJECTED WITH	WT. OF RIGHT MUSCLE	WT. OF LEFT MUSCLE	DIFF.	% OF ATROPHY
1	44	Control	0.930	2.427	1.497	61.7
2	44	Control	0.722	1.701	0.979	57.5
3	43	$\text{MgSO}_4\text{m}/1$	0.872	2.441	1.569	64.2
4	45	$\text{MgSO}_4\text{m}/1$	0.652	1.852	1.200	64.6
5	38	$\text{CaCl}_2\text{m}/4$	0.692	2.102	1.410	67.1
6	44	$\text{CaCl}_2\text{m}/4$	0.752	2.302	1.550	67.3
7	43	$\text{BaCl}_2\text{m}/10$	0.756	2.331	1.575	67.6
8	43	$\text{BaCl}_2\text{m}/10$	0.725	1.831	1.106	60.3
9	34	Control	1.075	2.159	1.084	50.2
10	33	Control	0.515	1.657	0.938	56.6
11	33	$\text{MgSO}_4\text{m}/1$	0.595	1.522	0.927	60.9
12	35	$\text{MgSO}_4\text{m}/1$	0.756	2.013	1.257	62.4
13	35	$\text{CaCl}_2\text{m}/4$	0.955	2.377	1.322	55.6
14	35	$\text{CaCl}_2\text{m}/4$	0.630	1.575	0.945	60.0
15	39	$\text{BaCl}_2\text{m}/10$	1.931	2.342	1.411	60.2
16	19	$\text{BaCl}_2\text{m}/10$	1.080	1.992	0.912	45.7
17	25	Control	1.554	2.821	1.267	44.8
18	25	Control	1.025	1.772	0.747	42.1
19	25	$\text{MgSO}_4\text{m}/1$	0.202	2.291	1.089	47.7
20	25	$\text{MgSO}_4\text{m}/1$	0.659	1.331	0.672	50.5
21	25	$\text{CaCl}_2\text{m}/4$	0.927	2.077	1.150	55.4
22	25	$\text{CaCl}_2\text{m}/4$	1.977	1.876	0.899	47.9
23	25	$\text{BaCl}_2\text{m}/10$	1.246	2.340	1.094	46.8
24	25	$\text{BaCl}_2\text{m}/10$	1.066	2.005	0.939	46.8

clusions of Langley and Hashimoto<sup>5</sup> that the curve of atrophy should fall more sharply in the third week and is generally lower than Langley and Kato's curve. The observations are too few to plot definite curves for each

salt. In each case the points indicated are the averages of two pigs similarly treated, except in the case of Pigs 15 and 16, which are plotted separately because Pig 16 died suddenly on the nineteenth day, immediately following injection, and the autopsy failed to reveal any sufficient, anatomic cause of death. It is possible that it was injected directly into a vein. Also in the case of Pig 13, the injections were stopped on the fifth day because of an extensive slough at the point of injection. In spite of these slight irregularities, a glance at the figure shows that the degree of atrophy is invariably greater in the injected pigs than in the uninjected animals.

While the results of these experiments are negative with respect to the question which they sought to answer; viz., can the fibrillary contractions of denervated muscle be stopped, and muscle wasting checked, by the subcutaneous injection of salts of the calcium group, there is still doubt as to whether the mode of treatment was such as to insure a sufficient concentration of ions in the atrophying muscles. It is possible that the intravenous injection of larger quantities of the salts would have been more effective. Experiments to this end have been undertaken by the senior author.

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# LABORATORY METHODS

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## A CONVENIENT, AUTOMATIC APPARATUS FOR EXPERIMENTS WITH SURVIVING ORGANS\*

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THE arrangements commonly used for studying the activity of excised organs in physiology and pharmacology are cumbersome, inefficient, and inconvenient. Frequently they lack convenient devices for proper control of temperature, washing the organ, etc. Anyone who has worked extensively with excised organs realizes the importance of controlling these factors promptly and efficiently in order that true and accurate results may be obtained. Therefore, it seemed desirable to construct an apparatus which would help to overcome these disadvantages and difficulties. The apparatus here described, it is believed, answers the requirements along these lines. The heating mechanism of this apparatus was suggested by a similar device used in a heart perfusion apparatus described by Fröhlich and Pollak.†

Briefly, the apparatus consists of an electrically heated brass chamber with inflow and outflow for the physiologic solution, and contains a small brass tube for admission of oxygen or air, a thermometer and an adapted heart lever for recording movements of the excised organ on a kymograph. The chamber is clamped on an iron support to which is screwed a pine board for attachment of a three contact switch and a resistance lamp, which maintains the temperature at a constant level of 38° C. In addition there is a glass leveling bulb for the physiologic (Tyrode, Locke or Ringer) solution, which is conducted to the inflow of the chamber by rubber tubing. The arrangements for heating, removal and replacement of the solution are practically automatic, requiring a minimum of time (about 1½ minutes) and effort for manipulation.

In detail, the apparatus consists of the following parts and their dimensions. *Chamber for excised organ*; this consists of a brass cylinder with tapering end into which is screwed a medium sized, metal stopcock. The diameter of the cylindrical portion is 3.8 cm. (1½ inches) and the length, 6.8 cm. (3½ inches). Its capacity is 50 c.c. to the mouth of the outflow tube soldered into and near the upper edge of the chamber. The outflow tube also serves as a handle for clamping the chamber on the iron support and consists of brass tubing 1 cm. in diameter and 14 cm. long. The wiring and insulation of the chamber are made as follows: First, the cylindrical portion of the chamber is covered with a layer of mica. Then, 48 feet of 27 gauge Advance wire are wound in a single layer over the mica, and on top of this is laid a thin sheet of asbestos. The entire covering is held in place by 2 brass bands, one at the top and one near the bottom of the cylinder. A post in each band serves for attaching wires from the resistance and main current. The aeration tube within the chamber is made from a small brass tube of 3 mm.

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Received for publication, February 16, 1922.

†Fröhlich and Pollak: Arch. f. d. ges. Physiol., 1920, 184: 14.

outside diameter and 1 mm. bore, and extends to the bottom. It is soldered to the inside wall of the chamber. The upper end of it bends over the edge of the chamber for attachment of rubber tubing conveying oxygen or air under pressure. *Lever*: this consists of an ordinary heart lever of the Harvard type with a small iron rod (10 cm. long), bent to a right angle at its distal end, the straight end being inserted in a socket in the frame underneath the axle. The lever is held in place by a small adjustable clamp on the iron support. The angle rod which is used for fastening the excised organ can be readily withdrawn from its socket and the lever used for other purposes. *Thermometer*: a short thermometer (about 15 cm. in length) is used and supported by 2 brass rings soldered inside

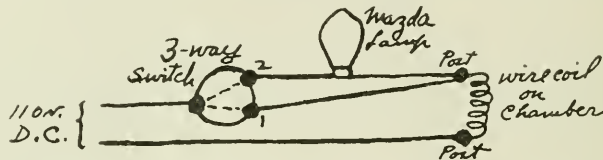


Fig. 1.—Diagram of Connections in Switch and for Resistance. At 1 the full 110 v. D. C. current is obtained; at 2, resistance, which maintains the temperature of the chamber at 38° C.

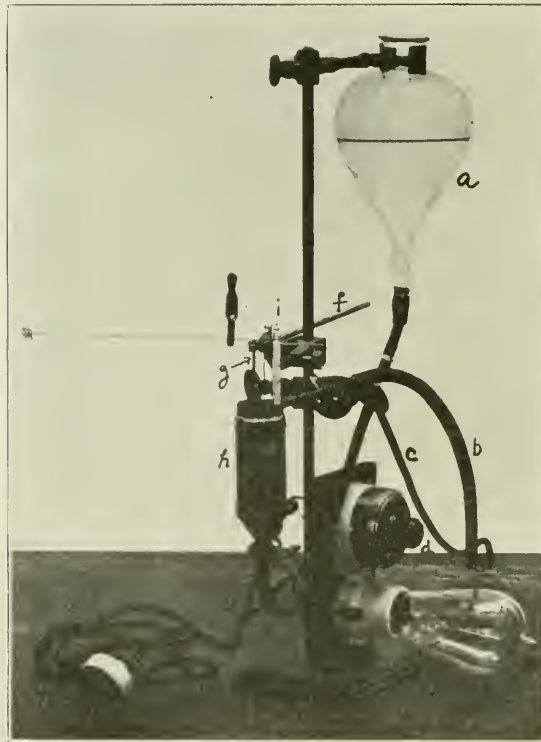


Fig. 2.—Photograph of the entire apparatus set up for use. (a) 1 L. Levelling bulb; (b) rubber tubing attached to outflow tube; (c) rubber tubing attached to aeration tube for oxygen or air; (d) 3-way switch; (e) 40-watt Mazda Lamp; (f) heart lever with (g) L-shaped rod for suspension of excised organ in (h) chamber; (i) thermometer, and (j) wires from socket and lamp to chamber.

the chamber. *Iron support*: an ordinary support about 45 cm. (18 inches) tall with a broad base suffices. *Glass bulb for the physiological solution*: this consists of a levelling bulb of 1 Liter capacity. *Resistance*: for this a 40 Watt Mazda lamp is used and attached together with a 3-contact switch to a small pine-board at the lower end of the support. A numbered 3-way switch is most convenient, giving the full 110 v. current at No. 1 and resistance at No. 2. A diagrammatic sketch of the wiring for the switch and resistance is presented in Fig. 1. Fig. 2 shows a photograph of the entire apparatus set up for use.



*Method of Operation:* Having filled the chamber with the physiological solution (Tyrode, Locke or Ringer), the switch is turned to No. 1, which gives the full current of 110 volts. The temperature in the chamber rises rapidly (in about 22 seconds) to 31° C. When it reaches 31° C. the switch is pressed to the next contact, i.e., No. 2. This connects with the resistance lamp. The temperature now rises more slowly, requiring about 1½ minutes to reach 38° C., and it remains at this level permanently. Variations in the temperature in the chamber may occur from variations in the electric current, and variable aeration. Aeration should be conducted at the rate of about 1 bubble per second. The strip of excised organ or tissue is introduced either before or after the temperature is raised. Removal of the solution is accomplished automatically by opening the stopcock at the bottom of the chamber and allowing it to flow through the handle, which serves as the outflow tube. The time necessary for removal of 50 c.c., i.e., one complete washing of the chamber, is about 20 seconds. In order to prevent toxicity effects from the chamber, when corrosion occurs, it may be nickelled or the inside coated with paraffin of high melting point (50° C.).

#### ADVANTAGES

The apparatus is simple, compact, easily transferable, permanent and inexpensive. It has been found very convenient for research and teaching. It has the following important advantages for mammalian tissues: warming of the organ or tissue is rapidly accomplished, and this facilitates the initiation and maintenance of functional activity and recovery from previous effects; overheating is practically impossible, and the organ is not exposed to the atmosphere during washing, or removal of solutions. The apparatus can also be used for amphibian tissues.

Thanks are due to Mr. Morris Dan of Cleveland for mechanical assistance in construction of the apparatus.

### A CONVENIENT FORM OF TEST TUBE RACK\*

BY STERNE MORSE, M.D., NEW YORK CITY

TEST tube racks in use have one practical disadvantage which is to a large extent overcome in the rack here figured. This is the necessity of removing a tube under examination in order that the whole of its contents, sediments, as well as supernatant liquids can be thoroughly examined. The type of rack here

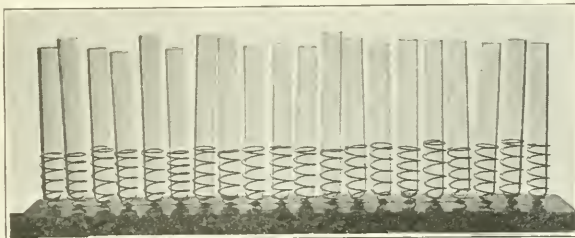


Fig. 1.

figured is so constructed, that every part including the bottom of the tube can be thoroughly inspected without touching the tube. It also has the merit of lightness, cheapness and ruggedness.†

\*Accepted for publication, April 27, 1922.

†This rack in several different types, can be obtained from Eimer and Amend, 218 Third Ave., New York City.

## WASSERMANN TEST TUBE AND PIPETTE WASHER\*

BY C. E. SWANBECK, M.D., CLEVELAND, OHIO

THE apparatus shown in the diagram was made from two nickel-plated brass tubes, which previously had served as towel racks. Ten small holes were drilled in each tube. The distance between each hole was made to correspond to the distance between the test tubes in the Wassermann rack. A five-

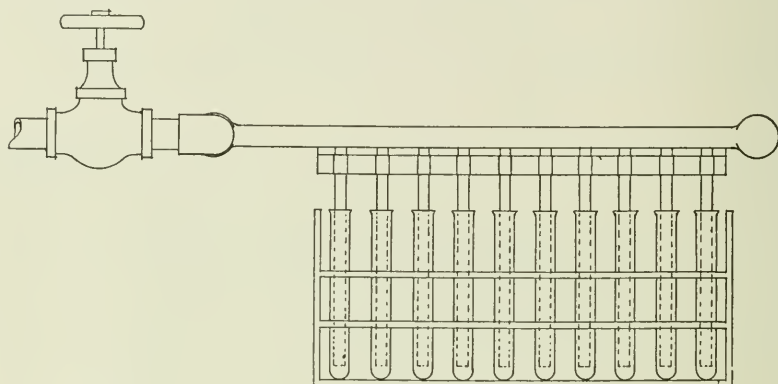


Fig. 1.

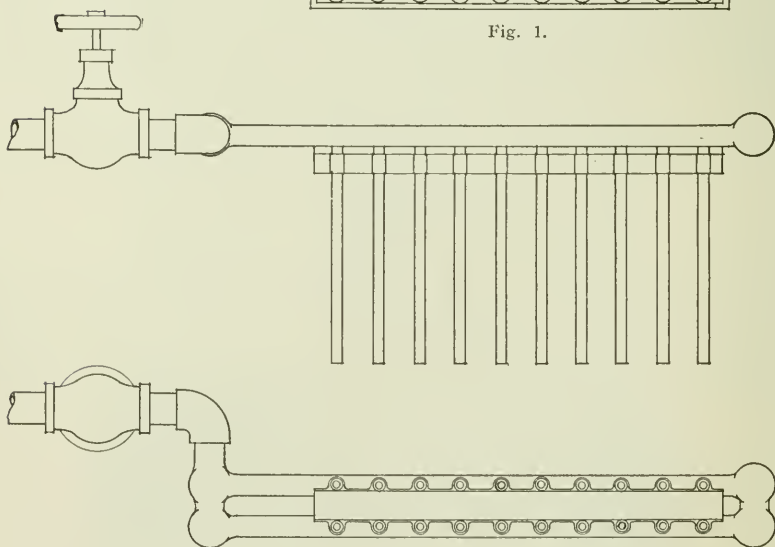


Fig. 2.

inch length of copper tubing with an outside diameter of  $\frac{1}{4}$  inch was now inserted in each hole and soldered in place. The two brass tubes containing the copper tubing were now united at each end with pipe couplings and held apart so that the copper tubes were in the same relationship to each other as the holes in the Wassermann racks. At one end was inserted a pipe fitting to

\*From the Laboratories of Mt. Sinai Hospital, Cleveland, Ohio.  
Received for publication, Dec. 12, 1921.

be attached to the general water supply pipe in the sink. In order to reinforce the copper tubing a wooden strip was inserted between the two rows of copper tubing and a narrow strip of copper tacked on in such a way that it held the copper tubing against the wooden strip.

When a rack containing Wassermann tubes is now attached to the above apparatus and the water turned on the tubes are thoroughly cleaned in ten minutes.

This outfit was devised because on testing some of our Wassermann tubes with benzidine a positive test for blood was occasionally found. When a large number of tubes are left to an orderly to wash, the washing is often super-

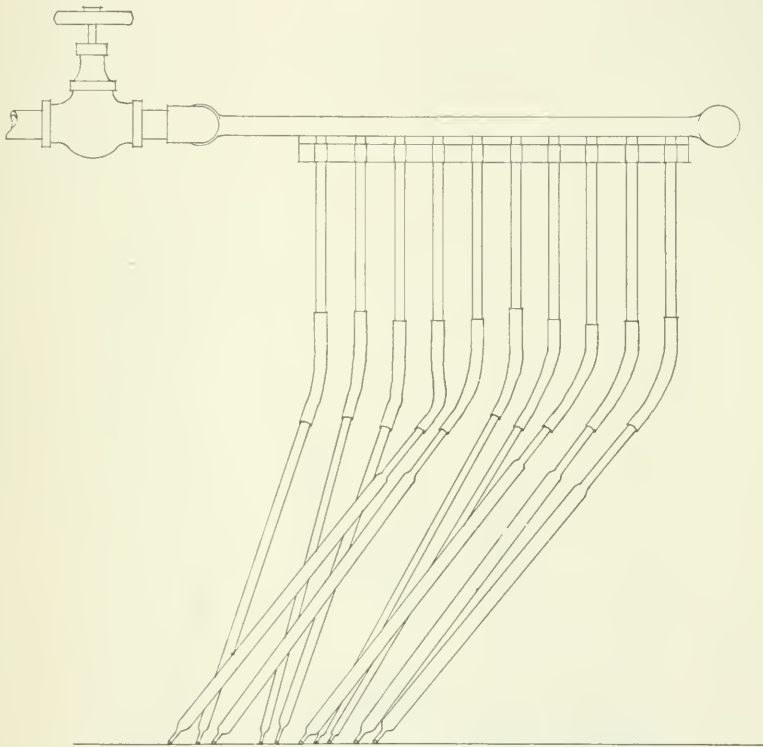


Fig. 3.

ficially done. With this outfit, the orderly places the rack of tubes as he receives them up to the washing apparatus and turns on the water supply, leaving the rack to wash for 15 minutes. Then the tubes are placed in distilled water to soak before drying and sterilizing.

By attaching short strips of rubber tubing to the ends of the copper tubing one can insert the serological pipettes into the rubber tubing and wash these in a similar manner. Twenty pipettes can be washed at one time on each washing outfit.

The advantages of this outfit are, (1) that the tubes and pipette are thoroughly clean, (2) there is considerable saving of time, (3) the washing can be left to an orderly.

A CONVENIENT APPARATUS FOR SIMULTANEOUS DETERMINA-  
TION OF TOTAL NONPROTEIN AND UREA NITROGEN, AND  
FOR PREVENTION OF BUMPING OF FILTRATE  
DURING BOILING\*

BY JOHN WALKER MOORE, B.S., M.D., AND LOUISE JONES, LOUISVILLE, KY.

THE estimation of total nonprotein and urea nitrogen in the blood has become nation-wide in the study of diseased conditions, since the publication in 1919 of Folin's "New Methods of determination of nonprotein nitrogenous products of the blood." The popularity of the methods as are recommended by Folin is embraced in the facts that they are less time consuming than methods heretofore employed, and that with a small amount of blood, a single filtrate



Fig. 1.

can be obtained in which the total nonprotein nitrogen and the various nonprotein nitrogenous products can be carried out with considerable accuracy. The technic of the methods recommended for the determination of the total nonprotein and urea nitrogen, simple though it seems, is not without a serious drawback, in that, bumping of the filtrate when heat is applied to the test tube is prone to occur, even though all precautions that are recommended have been rigidly

\*From the Medical Research Department University of Louisville.  
Received for publication, February 6, 1922.



observed. Particularly is this true in the distillation method recommended for urea determination. Here a single bumping usually invalidates the test, either by the filtrate going over into the receiving tube, or the acid of the receiving tube being sucked back into the filtrate.

Many ingenious procedures and contrivances have been advised from time to time, no one of which embraces simplicity and brevity and at the same time is absolutely successful.

The apparatus herein described, fulfills the requirement heretofore set forth, and has the advantage that the estimation of total nonprotein and urea nitrogen can be carried out successfully by one person, in the time allotted for a nonprotein nitrogen determination in the original method. The apparatus as shown in the photograph, is essentially that as recommended by Folin, with the addition that glass tubes (*a* and *b*) with deflected capillary points are inserted into test tubes. It will be noticed that these tubes are connected with rubber to the curved arms of a Y-shaped brass tube, whereas the straight arm of the Y is connected to a protected rubber bag for inflation. In the set up, test tube (*c*) is used for the total nonprotein nitrogen, and test tube (*d*) for urea nitrogen determinations. With filtrate in tubes, the rubber bag is inflated and a constant current of air is allowed to escape through capillary tip. A vigorous air current is not essential, it is therefore necessary to regulate air stream by use of rubber screw clamps (*e*). The flame is applied to each tube by means of a micro-burner, and the digestion and distillation of the filtrates are carried out simultaneously in the time and manner recommended by Folin. During the process of digestion, it is not desirable to continue the air current in the non-protein tube after the filtrate is about half evaporated, for at this stage of the digestion, bumping is not annoying, whereas if the current is allowed to continue, solidification will occur before oxidation is complete. It is not desirable, therefore, to have tip of tubing in test tube (*c*) extending farther than 1.5 cm. of bottom of tube. On the other hand, in the distillation method of determining the urea nitrogen, the capillary tip extends to within 0.5 cm. of bottom, as shown in test tube (*d*), and the air current allowed to continue throughout the test. This procedure not only promotes smooth boiling, but at the same time exerts a pressure which acts as a safeguard against suction that might take place during the determination. Using the method herein described, all annoying bumping is absolutely prevented. The flame can be cut down or increased at any time during the process of digestion or distillation without fear of disastrous results.

We have found the ordinary collapsible drinking cup a convenient and adequate protection for the flame of the microburner. In (*f*) the cup is collapsed, whereas in (*g*) the cup is opened.

## CHANGES IN NUTRIENT AGAR DUE TO CLARIFYING WITH EGG\*

BY LUTHER THOMPSON, URBANA, ILL.

WHILE working with lead subacetate agar it was noticed that the results of a previous period could not be duplicated, although the conditions in both instances seemed to be identical. By a process of elimination the only factor not common to both sets of experiments was determined to be the method



Fig. 1.—Centrifuged agar 24 hrs. incubation.

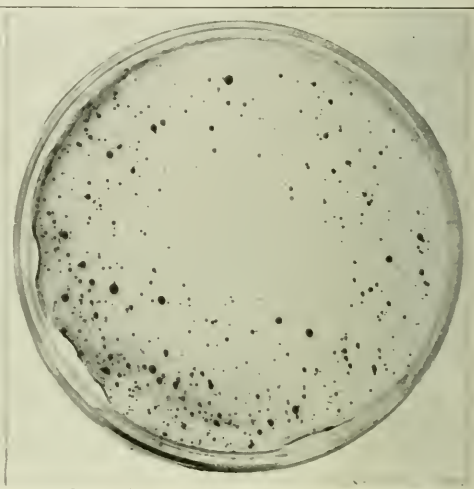


Fig. 2.—Agar cleared with whole egg 24 hrs. incubation.

*Bacterium typhosum*.

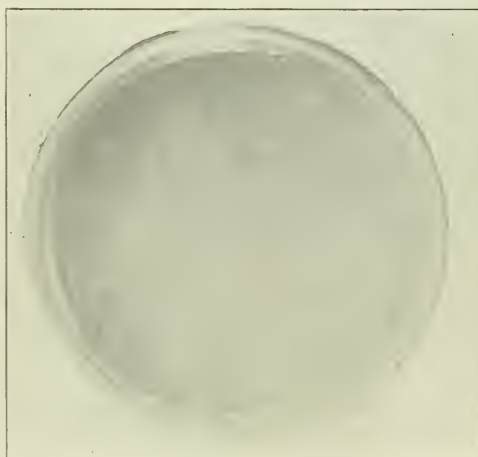


Fig. 3.—Centrifuged agar—24 hrs. incubation.

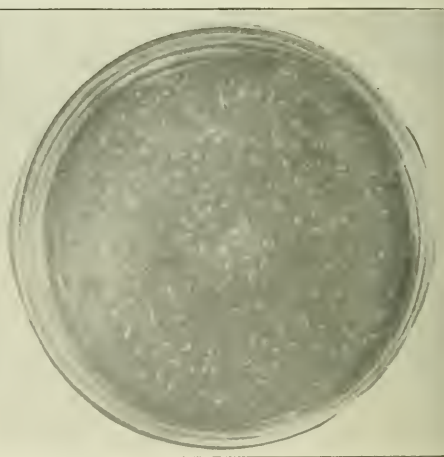


Fig. 4.—Agar cleared with egg white, 24 hrs. incubation.

*Bacterium communis*.

\*From the Department of Bacteriology, University of Illinois.  
Received for publication, February 13, 1922.

of clearing the agar, the first being an agar cleared by the addition of egg, and the second an agar cleared by centrifuging.

This suggested an inquiry concerning the effect upon agar of using egg or egg white as a clarifying agent. Accordingly two liters of agar were prepared as nearly as possible in the same manner except that one was clarified by the addition of two whole eggs, and the other by centrifuging. The reactions of both were made neutral to Bromthymol Blue and sterilized in the autoclave for 15 minutes at 15 pounds. To 100 c.c. of each agar was added 2 c.c. of a 2 per cent solution of lead subacetate. Tubes of each were then inoculated from an agar slant of *Bact. typhosum* and plates were made. Figures 1 and 2 show the results for the hydrogen sulphide test in the two kinds of media. This would indicate that egg adds to the agar certain protein compounds in which the sulphur is readily available, as for example cystin which is found in egg white.<sup>1</sup> Further tests made on agar cleared with egg white and egg yolk respectively, showed that such sulphur compounds may be derived from either the white or yolk of egg, and moreover, that when the yolk is fractioned to obtain hemato-gen and vitellin, these latter substances will add sulphur compounds to agar which are not coagulated by heat. In all cases where a part of the egg is used, lesser amounts of sulphur are added, which is further proof that the compounds containing it are not restricted to any one of the several proteins found in egg.

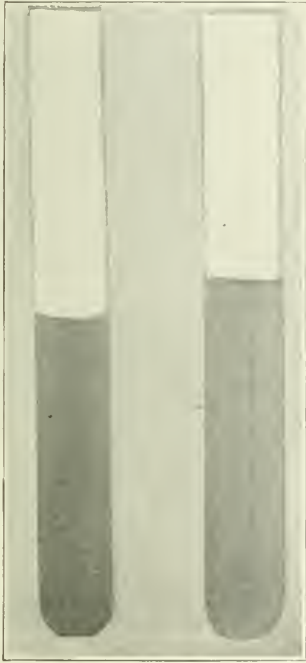


Fig. 5.—Tube at right—agar cleared with egg white. Tube at left—centrifuged agar. *Clostridium putrificum*—24 hrs. incubation.

The reaction of agar is made alkaline by egg white and acid by egg yolk. Using Bromthymol Blue as indicator, the following reactions were noted in three batches of agar just after clearing:

With egg white .....	$P_H$	7.2
With egg yolk .....	$P_H$	6.4
With whole egg .....	$P_H$	6.8

In each case an equal amount of egg substance was used and the reaction read from color standards.

A fact seemingly of greater significance than those previously mentioned is that of the addition of glucose to agar from the white of egg. Figs. 3 and 4 show the comparison between centrifuged agar and agar cleared with egg white. Both were inoculated with *Bact. communis*, but the one made with the egg agar shows numerous gas bubbles throughout, thus showing the presence of a fermentable substance. This finding of sugar in such decided quantity in the agar suggests that such a medium might be more favorable for anaerobic

culturing than centrifuged agar, and such appears to be the case. Fig. 5 shows two deep stab cultures of *Clost. putrificum*. After 24 hours' incubation the egg agar shows an abundant growth along the inoculation, while the other shows but little growth. The reactions of the two media were practically the same. Other tests made in the same way confirmed the first result. The egg agar also gives somewhat larger surface colonies than the centrifuged agar in 18-24 hours on petri dish cultures with *B. communis* and *Bact. typhosum*.

The Committee on the Descriptive Chart<sup>6</sup> has permitted the use of egg white as an alternative method of clarifying gelatine. If the results given above are true this would produce a medium considerably different from one obtained by filtering or centrifuging.

#### CONCLUSIONS

In making a standard agar the method of clarifying should be specified. An agar which is egg-cleared is a special, or egg agar because:

1. It contains sulphur compounds derived from the egg.
2. The reaction of the agar is influenced.
3. A fermentable substance is added, presumably glucose from egg white, which in both anaerobic cultures and on aerobic plates affects the rate of growth of certain bacteria.

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## AN EASY METHOD OF OBTAINING SAMPLES OF URINE FROM THE MALE DOG\*

BY FRANK C. MANN, M.D., ROCHESTER, MINN.

TWO methods are usually employed for obtaining urine from the dog; one by keeping the animal in some form of metabolism cage where the excretions are caught, and the other by catheterization. The former method requires a specially constructed cage and there is always the possibility of contamination of the urine with fecal material. The latter method is only applicable to the female as routine catheterization is not feasible in the male dog unless urethrotomy has been performed. Repeated catheterization in either the female or male dog after urethrotomy is often followed by cystitis even though good technic is maintained. A satisfactory procedure is as follows:

The animal is kept in a cage since there is then a greater tendency to al-

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\*From the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minn.  
Received for publication, March 8, 1922.



low the urine to collect in the bladder. A Hoffman screw clamp is placed on the prepuce in a manner that will just prevent the escape of urine. To obtain the specimen in the average animal remove him from the cage and give him his freedom, preferably in the run where he takes his daily exercise. Usually in a very short time he will urinate into the sheath of the penis. By gently loosening the screw clamp the urine can be collected in a container. Specimens can thus be obtained at one to two-hour intervals throughout the day.

The method is not applicable if the total amount of urine is desired at a definite time, as the bladder may not be completely emptied, and also bacteriologic studies of the urine cannot be made. If specimens only are desired, the procedure requires less time, less assistance, and less inconvenience to the animal than catheterization. By this method it is possible to obtain specimens of urine from a dozen male dogs in half an hour's time.

If it is desired to drain the bladder constantly, a ureteral catheter which is easily passed in the male dog, is inserted so that one end lies in the bladder and the other in the cavity of the sheath. The screw clamp is then applied to the prepuce. Urine will drain from the bladder through the ureteral catheter into the cavity of the sheath practically as fast as it is secreted, from which it can be drained every hour or two.

The average animal usually does not pay the slightest attention to the clamp and if care is exercised in its adjustment it may be left in position for many hours without inconvenience to the animal. It should be emphasized that the procedure does not cause pain.

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## A BOX FOR HANDLING SMALL ANIMALS IN THE LABORATORY\*

By LINWOOD D. KEYSER,† M.D., ROCHESTER, MINN.

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FOR the past two years in the Laboratory of Surgical Research of the Mayo Foundation we have used a simple box for controlling small laboratory animals such as rabbits and cats, so that tubation, feeding, and intravenous injections into ear veins can be carried out easily by one person.

The box is oblong, its dimensions being 42.5 by 22.5 by 22.5 cm. It is divided into two segments, which are hinged (Fig. 1). In each end is a circular aperture, one 6 cm. in diameter, and the other of 5.5 cm. in diameter. When the box is open the apertures are divided into semicircles, two in the upper and two in the lower half of the box. The two apertures offer a range of size suitable for the heads of most rabbits.

The animal (rabbit or cat), is placed in the box, and the box closed with the head of the animal protruding through the aperture, pillory fashion. A hinge holds the box closed (Fig. 2). The animal's head is held relatively fixed and the investigator is protected from scratching. For esophageal intubation

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\*Received for publication, April 22, 1922.

†Fellow in Pathology, Mayo Foundation.

a No. 10 French soft rubber catheter is introduced through a small opening in a wooden tongue depressor which has been pried between the teeth of the animal as a mouth gag. The catheter is passed into the esophagus by touch and to the stomach. Fluids or suspensions may thus be introduced into the stomach as shown in Fig. 2.

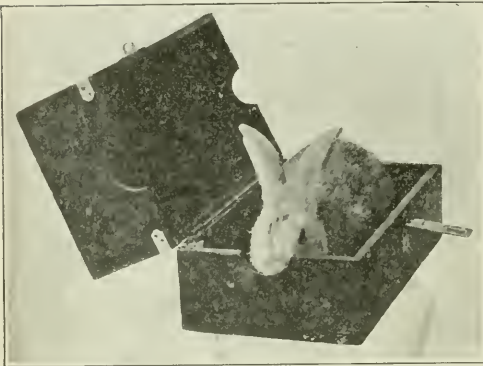


Fig. 1.—Box open showing relation of parts.



Fig. 2.—The box in use showing manner of introducing fluid through tube into the stomach. The animal suffers no pain.

For the intravenous injection of bacteria, drugs, and so forth, the ear is caught at its tip and the head is gently but firmly pulled forward away from the box. The hypodermic needle is then introduced into the marginal vein and the injection made.

Animals handled in this manner are subjected to a minimum amount of discomfort.

# *The Journal of Laboratory and Clinical Medicine*

VOL. VII

ST. LOUIS, SEPTEMBER, 1922

No. 12

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Washington, D. C.

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## EDITORIALS

### *Internal Secretion*

ENDOCRINOLOGY has reached the apogee of its popularity as a vehicle for extravagant hypotheses. At a time when the ductless glands are being referred to in a semiscientific publication as the "glands regulating personality," when periodicals and three volume treatises are devoted almost exclusively to the literature of endocrinology, when the subcutaneous injection of extracts from ciliary bodies is recommended for the treatment of iritis and of extracts of heart muscle for myocardial disease, when one reads that the internal secretory glands "already unfold before the astonished gaze of the seeing eye a land of promise beside which the vast territories conquered by Lister and Pasteur are destined to pale into honorable insignificance," at such a time one is refreshed and the mind becomes clearer on reading a vigorous protest from the pen of a physiologist who has been a recognized leader in the study of the internal secretions. Swale Vincent,

professor of physiology in the University of London, in an address before the Royal College of Surgeons has made a critical examination of current views on internal secretion. (Lancet, London, August 12, 1922.)

Theophile de Borden in 1775 first promulgated the theory that each organ in the body secretes into the system a special substance which acts on the other tissues of the body. This held for all organs no matter what their nature. J. Müller in 1838 discussed those glands without ducts and believed that they exerted a certain plastic influence on the fluids circulating through them. Berthold of Göttingen first demonstrated experimentally the existence of an internal secretion in 1849, by removing testes from young cockerels and transplanting them to the surface of the intestine. The doctrine of internal secretion as it is generally taught today was founded by Claude Bernard and Brown-Séquard.

The study of internal secretion has consisted chiefly in observing the effects of injection of organ extracts into experimental animals. The reactions observed are pharmacodynamic only and do not necessarily express the physiologic activity of the organ under consideration. The existence of these active substances found experimentally does not necessarily prove that the organs from which the extracts were made possess an internal secretion or that that secretion acts in the economy in the same manner in which it acts in the laboratory. Thus it has been found that an extract from the posterior lobe of the pituitary body of a bull will cause contraction of the uterus of a female guinea pig. It is quite impossible to visualize such a function existing in the animal from which the extract was made. The observations are, to be sure, of great pharmacologic interest but it is not until after many other lines of research have been followed that one would be justified in drawing conclusions as to the presence and action of an internal secretion.

Early investigators in the field of endocrinology found that an extract made from the medulla of the adrenal bodies, when injected into animals, caused certain characteristic reactions, chiefly of a pressor nature and affecting principally the heart, blood vessels and other types of muscle. The same was found to be true of extracts from that portion of the pituitary body which is of nervous origin. As a result of these and similar observations an extravagant theory was built up to the effect that many or most of the bodily functions are controlled by the balancing of pressor substances secreted from these two sources and certain antagonistic or depressor internal secretions formed elsewhere in the body. In spite of all the work that has been done in recent years on the physiology of the adrenal bodies we know as yet little concerning their function. The function of the cortex, the larger portion of the gland, is unknown. There can be little doubt that the medullary portion actually secretes small amounts of epinephrin into the circulation. The importance of this substance to the general physiology is by no means as certain. Vincent goes so far as to state that there is little or no evidence that the medulla of the adrenal bodies is of any importance in the normal economy of the animal. He believes that notwithstanding the views of Cannon it is doubtful whether the adrenalin output is at all affected by emotional excitement.



The prevailing hypothesis of thyroid function is based on sound chemical and pharmacologic research and on more convincing clinical evidence than holds for the adrenal gland. Thyroxin, isolated by Kendall, acts through the blood stream as a catalyzer for the production of energy within the tissues. Thyroxin causes no change in the chemical reactions within the body but merely increases the rate of reaction. According to Kendall the thyroid gland has been added to the animal organism to permit a greater flexibility of energy output. Altered function of the thyroid gland is fairly easily recognized and treatment is fairly successful whether the alteration be in the nature of hyper-function or hypo-function. However the diagnosis of hyper-function or hypo-function is often made on insufficient evidence and when the actual pathology is elsewhere.

From knowledge of the pharmacology of pituitary extracts, and from a study of diseases associated with abnormalities of that organ, we have some fairly definite information regarding its activity. The problem is however complicated by the fact that in all probability pituitary function is interrelated with the function of certain other endocrine glands. Moreover some of the recent work indicates that many or all of the abnormal findings usually attributed to a lesion of the pituitary body are in reality the result of damage to the base of the brain in the region of the sella tureica and not to disease of the hypophysis itself. In clinical work there is a well recognized tendency to class all growth anomalies as pituitary in origin and the term "dyspituitarism" has acquired a diagnostic popularity which the facts do not warrant.

Recent work indicates that the parathyroids control muscle tone by keeping down the amount of some toxic substance, possibly guanidin, in the circulating blood. This is accomplished either by checking its production or by bringing about its conversion into creatin. There is a tendency to classify all "tetanic" disorders as due to lesions of the parathyroid glands.

According to Vincent our knowledge of the internal secretion from the pancreas and the gonads is based chiefly on clinical rather than on experimental observations. However, the epochal work of Banting and Best reported in this Journal in February and May, 1922, in which they have a pancreatic enzyme capable of splitting sugar and diminishing glycosuria and hyperglycemia, promises to be one of the most valuable, recent discoveries. The value of such an extract in therapeutics will easily equal that of thyroid extract.

We know nothing definite concerning the function of the thymus.

In spite of paucity of actual knowledge of the function of the internal secretory glands, the pharmaceutical market is virtually flooded with preparations made from extracts of the various body organs. It seems logical to conclude that these substances would not be manufactured in such abundance if they did not find a ready market among practicing physicians. Practically all are without doubt quite inert when taken by mouth. The active substance from the thyroid gland is possibly the only one that exerts any influence when so administered.

Organotherapy is as old as is the art of medicine, and the superstition

which impelled the savage to devour the heart of the lion so that he might acquire the courage of that animal is the same superstition which will impel a physician to prescribe prostatic extract for disease of the prostate gland or senility or for impotence. Celsus and Dioscorides recommended the use of various animal organs for the relief of symptoms considered to be due to impaired function of those same organs in man. Cushing suggests that "The Lewis Carroll of today would have Alice nibble from a pituitary mushroom in her left hand, and a lutein one in her right, and presto! she is any height desired."

Vincent does not deny the undoubted importance of endocrine function in physiology, but he voices his protest at the indiscriminate and uncritical application or misapplication of the meagre knowledge at our disposal. "There is indeed serious danger that the nucleus of sound knowledge which justifies a belief in the doctrine of internal secretion may be jeopardized by a too drastic destructive criticism, in the same kind of way that, when phrenology was exploded by Flourens in 1822, brain physiology was so seriously injured in the fray as not to recover for close upon half a century. One hears cynically inclined onlookers and medical scoffers suggesting at the present time that we should encourage the vitamins in order to keep down the plague of endocrines, and prophesying that sooner or later some new fad will be welcomed whose function it will be to relieve the strain of vitaminology."

—W. T. V.

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### *Activities of the Medical Section of the League of Nations*

WHATEVER may be the condition of other sections of the League of Nations, the Medical Section evidently is far from being moribund. In December, 1921, a committee of this Section met in London for the purpose of taking steps for the standardization of antitoxins and other biologic products. So far as diphtheria is concerned, the units in different countries are not materially unlike, but in the case of tetanus there are great differences, and it is proposed that committees with members from various countries try to establish standards for these agents. A proposition has been made that sailors be treated free of charge in any country they might be when needing treatment. This is especially desired insofar as treatment for syphilis is concerned. Another thing that the Medical Section of the League of Nations is attempting to do is to standardize the Wassermann Test; also to standardize or define arsenical preparations used in the treatment of syphilis. These certainly are commendable undertakings.

In May, 1922, the Medical Section of the League of Nations held a meeting in Paris and discussed some most important health and medical subjects. In 1920 an international congress was called in Paris for the purpose of establishing an international list of the causes of death. This work has never been completed and the list has not been published. At this May meeting in Paris the Medical Section of the League of Nations took

steps to complete this list of causes of death and to secure immediate publication. At the same meeting the opium question was discussed from all angles. An Englishman from India held that in that country opium is taken as tea or tobacco is taken in other countries. It is a stimulant for workers engaged in arduous labor, for soldiers who have to make long marches, and even for animals. He admitted that its employment might have injurious effects on the young, but that it is not harmful after forty years of age when the vital energy is on the decline. Unlike cocaine, it does not tend to increase crime. Insurance companies do not distinguish between those who take and those who do not take opium. In thousands of villages opium takes the place of medicines for all kinds of sickness, and it also plays a part in certain religious practices with which it would be dangerous to interfere. This seems queer, and it is rather difficult for us to believe. It is highly proper that the Medical Section of the League of Nations refused to endorse this statement and decided on further investigation.

At the Paris meeting it was proposed that students of epidemiology in one country be invited into other countries and given opportunity therein to continue their studies. This would be of the greatest value to epidemiology. This is a subject which needs to be studied in its world-wide aspects and it would be of great advantage if French or English students of epidemiology could go into Russia and study, together with the native epidemiologists, the diseases now prevailing in that country. The Section passed the following resolution: "The Health Committee, taking note of the proposal regarding an interchange of sanitary staff between different countries interested in its being carried into effect, esteeming it of the greatest utility for sanitary officers to become acquainted with practical solutions of public health problems as well as with the organization of public health services in various countries, recommends that the necessary steps should be taken by approaching the governments interested in the matter in order to carry into effect this interchange, at least as a provisional and experimental measure."

It was deemed desirable that the appointment of a committee consisting of experts nominated by the Mandatory Powers in Africa should collect information available regarding the prevalence of sleeping sickness and tuberculosis among the native population of Equatorial Africa and the measures recommended to prevent the extension of these diseases in this region should be determined.

Steps were taken for the strengthening of international arrangements for the prevention of epidemic diseases in certain areas of the Near East and in connection with the Mecca pilgrimages. It seems that nothing in an international way has been done in this matter since 1912. Pilgrimages in the near future may scatter disease far and wide over the three continents of the Eastern Hemisphere and possibly may extend the distribution of these viruses to the Western Hemisphere. It is pleasing to note that, notwithstanding our country is not a member of the League of Nations, the Medical Section of that body has invited Dr. Josephine Baker, Director of the Bureau of Child Hygiene in New York City, to become a member and

to participate in their deliberations. This is a recognition of the splendid work Dr. Baker has done in New York, and we are sure that the medical profession throughout the United States will agree that she well deserves this distinction. The work of the Epidemic Commission of the League is the first instance of international effort in public health. It differs fundamentally from the activities of various relief organizations, inasmuch as it is supported entirely by the governments concerned and is in no sense a charitable organization. The Epidemic Commission has not undertaken field work of its own, but has acted through the public health administration of the countries concerned. It has declined to have any relation with the relief organizations except through the intermediary of its government. It bases its work on the necessity of strengthening the public health and sanitary organization in the country where it is needed. Relief organizations as a rule are interested in the immediate alleviation of sufferings, while the Epidemic Commission looks forward to more permanent results. The epidemic situation in Europe, especially in Russia, will continue to be serious for years to come and it is certain that the Epidemic Commission of the League of Nations will have to function for a long time. If the developments in the scheme of the economic reconstruction of Russia should allow of sufficient funds being collected through the government the Epidemic Commission should be the medium for putting into effect any such scheme. It was suggested at the meeting that steps be taken to facilitate the methods of securing cheap supplies of quinin and arsphenamine in all the countries of the world.

—V. C. V.

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### *Cultivation of the Gonococcus*

ERICKSON and Albert<sup>1</sup> have tested out the various media on which the gonococcus has been grown and conclude that testicular blood agar with a reaction of  $P_H$  7.4-7.8 is the most favorable medium for the isolation and subsequent cultivation of this organism. Beef testicle, from which all connective tissue has been removed, is put through a meat grinder and infused overnight with twice its weight of distilled water. The next morning the mixture is heated to 50° C., allowed to stand for one hour, and then brought to the boiling point. After standing for another hour in order to allow the solid particles to settle, the supernatant fluid is decanted and used for the preparation of culture media. To this infusion, 2 per cent peptone, .5 per cent glucose, .3 per cent monobasic sodium phosphate, and 2.5 per cent powdered agar are added. This mixture is heated until the agar is dissolved, then treated with phenol red and the reaction adjusted to the above-given figures. The medium may then be tubed and autoclaved for twenty minutes at fifteen pounds. The titration may be checked after sterilization. While the contents of the tubes are still liquid, human blood is added in the proportion of .5 to 2.5 per cent. If human blood is not at hand defibrinated rabbits' blood (1 to 5 per cent) may be employed.

—V. C. V.

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<sup>1</sup>Jour. Infect. Dis., 1922, xxx, 268.



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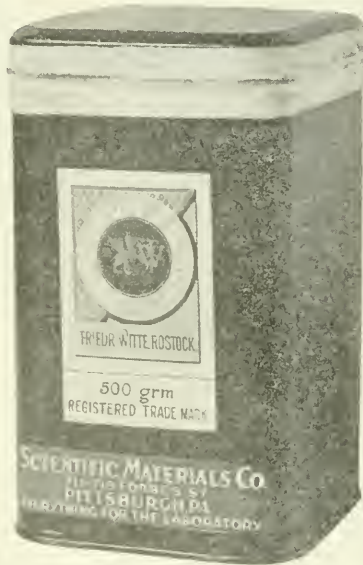
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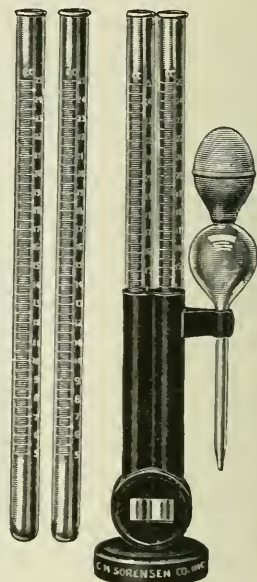
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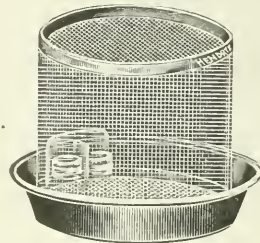
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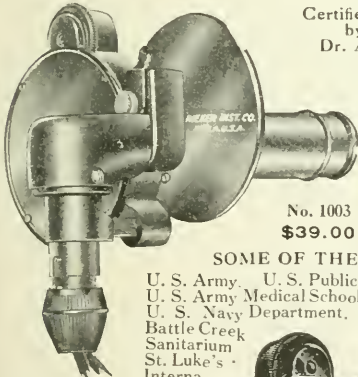
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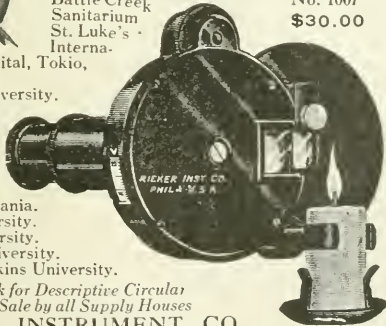
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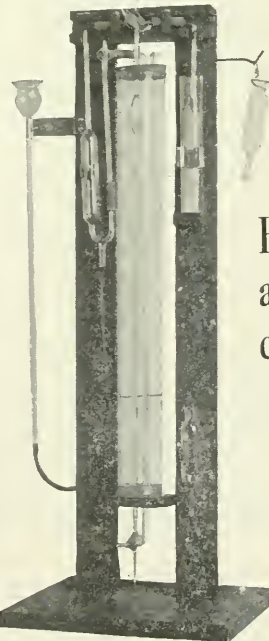
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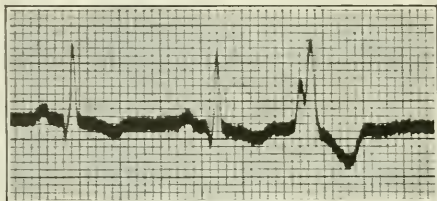
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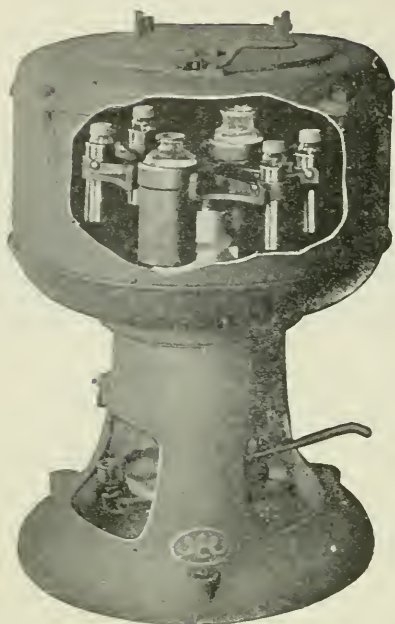
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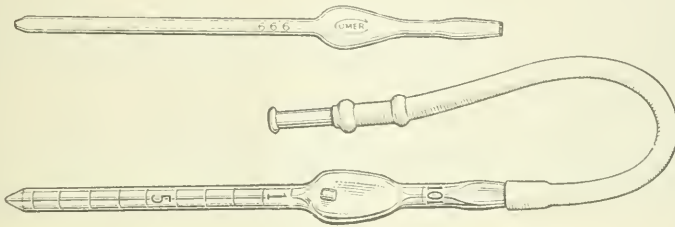




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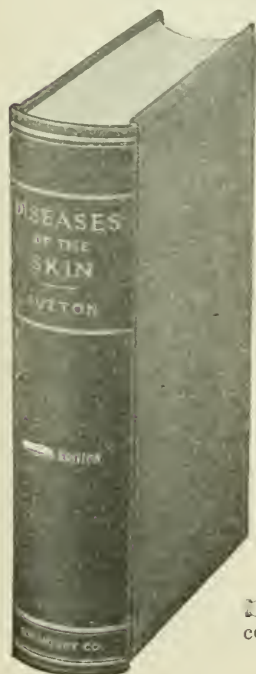
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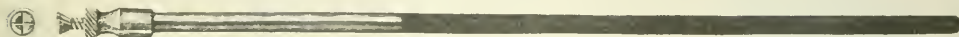
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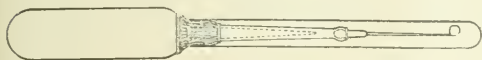
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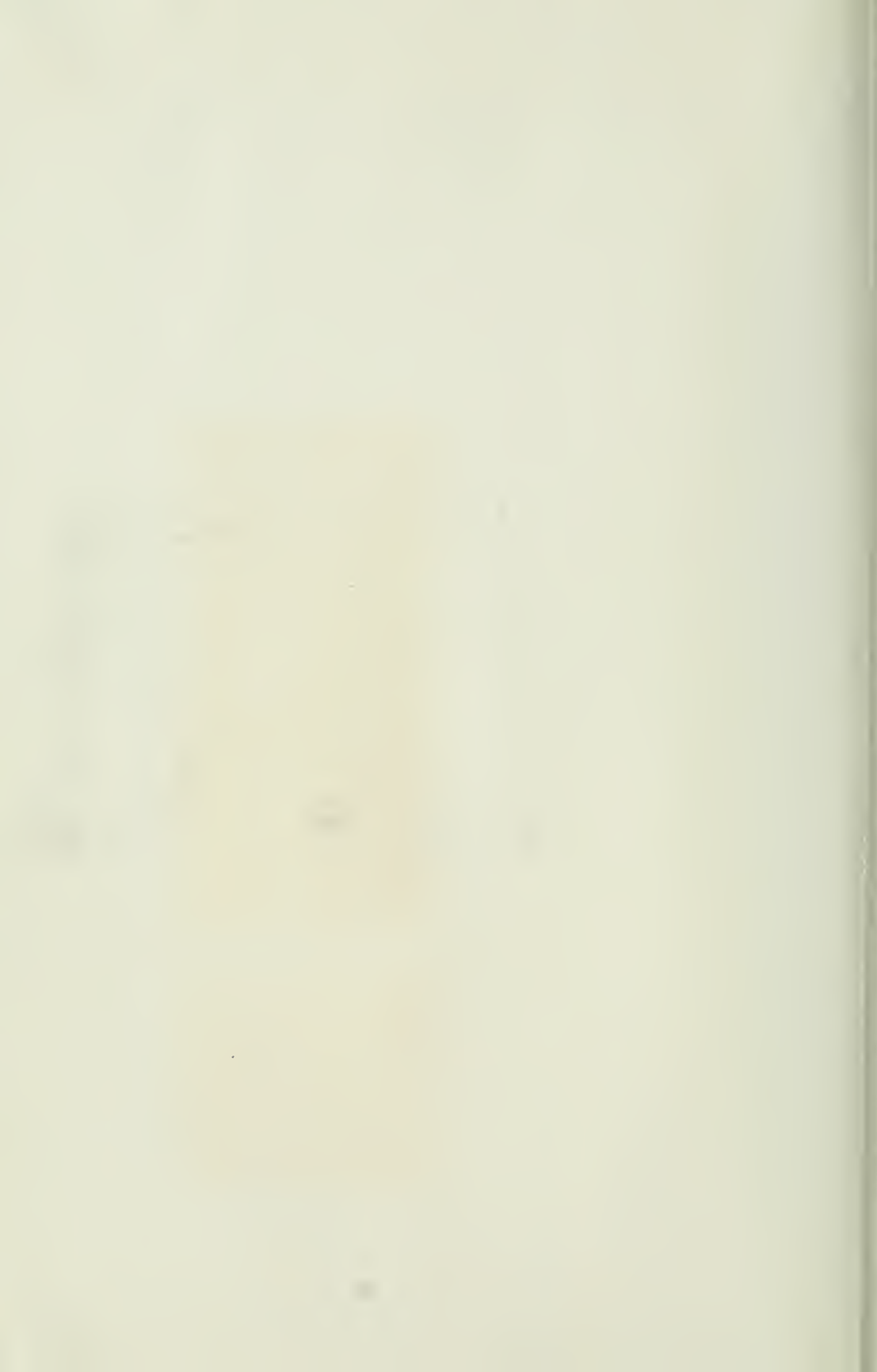
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